THE

Progressive Fish-Culturist

A Quarterly for Fishery Biologists and Fish-Culturists

Volume 44 January 1982 Number 1

CONTENTS

Movements of Hatchery-reared Rainbow, Brook, and Brown Trout Stocked in a Virginia Mountain Stream. *L. A. Helfrich* and *W. T. Kendall* 3

Evaluation of Tilapia Meal for Fish Diets. *J. W. Foltz, J. M. Gibson,* and *J. T. Windell* 8

Furunculosis in Brook Trout: Infection by Contact Exposure. *R. C. Cipriano* 12

Inhibition of Spermatogenesis and Vitellogenesis in Rainbow Trout by Hormonal Additives in the Diet. *R. Billard, M. Richard,* and *R. Rombauts* 15

Ammonia Removal by Clinoptilolite in the Transport of Ornamental Freshwater Fishes. *C. E. Bower* and *D. T. Turner* 19

Range Pellets as Supplemental Crayfish Feed. *S. Cange, M. Miltner,* and *J. W. Avault, Jr.* 23

Manuring Rates for Production of Blue Tilapia in Simulated Sewage Lagoons Receiving Laying Hen Waste. *R. B. McGeachin* and *R. R. Stickney* 25

Common Carp Eggs as a Starter Diet for the Intensive Culture of Smallmouth Bass Fry. *D. W. Willis* and *S. A. Flickinger* 28

Central System for Monitoring the Quality of Water from Many Sources. *B. P. Kinghorn* 30

Rearing Lake Whitefish to Fingerling Size. *G. R. Raisenan* and *D. J. Behmer* 33

Feeding Success of Hatchery-reared Kokanee Salmon when Presented with Zooplankton Prey. *G. L. Vinyard, R. W. Drenner,* and *D. A. Hanzel* 37

Seasonal Distribution of Esocids in a Power Plant Cooling Reservoir. *D. T. Henley* and *R. L. Applegate* 40

Shielded-needle Technique for Surgically Implanting Radio-Frequency Transmitters in Fish. *M. J. Ross* and *C. F. Kleiner* 41

Effects of a Diet of Bullfrog Larvae on the Growth of Largemouth Bass. *E. J. Evenson* and *K. C. Kruse* 44

Comparative Hatching Success of Lake Trout Eggs in Lake Michigan Water and Well Water. *C. C. Edsall* and *M. J. Mac* 47

Water Flow Controller. *B. P. Kinghorn* 48

Holding Atlantic Menhaden in a Closed System for Environmental Research. *S. J. Hope* 51

Effect of Delivery Method and Antigen Preparation on the Production of Antibodies Against *Aeromonas hydrophila* in Channel Catfish. *R. L. Thune* and *J. A. Plumb* 53

Further Observations on a Fish Production System that Incorporates Hydroponically Grown Plants. *R. J. Sutton* and *W. M. Lewis* 55

Some changes to the instructions for contributors have been instituted with this issue of *The Progressive Fish-Culturist* (see inside of back cover).

Movements of Hatchery-reared Rainbow, Brook, and Brown Trout Stocked in a Virginia Mountain Stream

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ABSTRACT: During May 1979, 1,061 catchable-sized, hatchery-reared rainbow trout (*Salmo gairdneri*), brook trout (*Salvelinus fontinalis*), and brown trout (*Salmo trutta*) were tagged and stocked in pool and riffle habitats in Big Stony Creek, Giles County, Virginia. Of this total, the movement patterns of 275 recovered trout were determined through voluntary tag return and creek census information. Most (75%) of the trout were recovered within 1 km of their respective release sites; 16% remained within the initial stocking locations. Most of the recaptured brook trout (69%) and rainbow trout (59%) were recovered downstream, whereas brown trout (69%) moved primarily upstream. The median dispersal distances for brook trout (195 m downstream) and rainbow trout (60 m downstream) were not significantly different from one another, but were significantly different ($P < 0.001$) from that of brown trout (90 m upstream). Although trout stocked in pools generally exhibited less movement than those stocked in riffles, the type of stream habitat into which the trout were introduced had no significant effect on the distance or direction of dispersion of the three species.

Stocking programs for catchable trout represent a major component of coldwater sportfisheries management in the United States. Each year more than 70 million trout of catchable size, produced at a cost of about $18 million, are stocked to provide recreational fishing opportunities for an estimated 18 million anglers nationwide (U.S. Fish and Wildlife Service 1968, 1976, 1977). The principal objective of such programs is to maximize the return of stocked trout to the angler. Since stocked trout that move out of authorized public fishing waters or into inaccessible stream areas constitute a significant recreational and economic loss, the anticipated dispersion patterns of hatchery-reared trout and the factors that influence movement are important management considerations.

Published descriptions of the movements of catchable-sized, hatchery-reared trout are provided by Stefanich (1951), Ratledge and Cornell (1953), Bjornn and Mallet (1964), and Moring and Buchanan (1978) for rainbow trout (*Salmo gairdneri*); by Hoover and Johnson (1937) and Smith (1941) for brook trout (*Salvelinus fontinalis*); by Hazzard and Shetter (1938) and Newell (1957) for rainbow and brook trout; by Klein (1974) for rainbow and brown trout (*Salmo trutta*); and by Cobb (1934) for brown and brook trout. Only Trembly (1943) and Cooper (1952) have reported on the movement of brook, brown, and rainbow trout stocked simultaneously in the same stream habitat. These two studies, conducted on low-gradient streams in Pennsylvania and Michigan, indicated that although most trout stocked were sedentary, brook and rainbow trout stocked in the spring exhibited a tendency to move farther and downsteam, whereas brown trout tended to move less and generally in an upstream direction.

Comparatively little is known about the dispersion habits of these three species when they are stocked concurrently in relatively small, high-gradient mountain streams that are characteristic of Virginia's catchable trout waters. The purpose of the present study was to describe and compare the disperison patterns of hatchery-reared brook, brown, and rainbow trout stocked in Big Stony Creek, Giles County, Virginia.

Study Area

Big Stony Creek, a tributary of New River, lies within the ridge and valley physiographic province of the Allegheny Mountains in the southwestern part of the State. This second-order stream is about 23 km long and drains an area of 126 km^2; it is characterized by a steep gradient (21 m/km average fall), a coarse substrate (largely gravel and boulders), and abundant riparian vegetation. Streamflows ranged from more than 6.5 m^3/s in March to a minimum of 0.4 m^3/s in August. Stream temperatures ranged from 4°C in March to 18°C in July. At the time of stocking during the second week in May, stream flow averaged 3 m^3/s,

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stream temperature averaged 12°C, dissolved oxygen levels were characteristically high (95% saturation), and the pH was slightly alkaline (7.4). Resident fishes observed in Bg Stony Creek included the creek chub (*Semotilus atromaculatus*), blacknose dace (*Rhinichthys atratulus*), longnose dace (*R. cataractae*), mountain redbelly dace (*Phoxinus oreas*), rosyside dace (*Clinostomus funduloides*), northern hogsucker (*Hypentelium nigricans*), white sucker (*Catostomus commersoni*), mottled sculpin (*Cottus bairdi*), and fantail darter (*Etheostoma flabellare*). The stream also contains a very small population of wild, naturally reproducing brown trout.

About 14 km of Big Stony Creek lie within the Jefferson National Forest. The stream parallels Virginia State Route 635; it is heavily stocked and intensively fished because of its easy access and public ownership. The study site consisted of a stream section 5.5 km long, bounded downstream by the U.S. Forest Service's Interior Park and upstream by a bridge on Forest Service Road 942.

Methods

The study area was partitioned into 183 separate 30-m sections. The upper and lower boundary of each section was prominently marked with numbered plastic flagging for the identification of capture sites. On two dates (7 and 10 May 1978), a total of 1,061 trout were stocked in nearly equal numbers in three representative riffle and three pool habitats. These pools and riffles were selected at random from those which appeared to be most representative of the stream and were located near the center of the study area. Mean length and depth of the selected pools (16 and 1.5 m, respectively) and riffles (18 and 0.4 m, respectively) appeared adequate to hold trout if they elected to remain at the release sites. The trout were stocked at the midpoint in each of the selected pools and riffles. All trout were obtained from the Coursey Springs State Fish Hatchery and marked with individually numbered Floy internal anchor tags (Dell 1968). Lengths and weights of 20 randomly chosen trout of each species averaged 27.3 cm and 236.7 g (rainbow trout), 27.1 cm and 249.3 g (brook trout), and 27.8 cm and 232.1 g (brown trout).

Data on trout movement and harvest from Big Stony Creek were collected primarily by a creel survey of fishermen. Creel surveys have been shown to be an effective method for determining the movements of fish (Stefanich 1951). All fishermen who were encountered were interviewed; preprinted creel forms were used. Items of primary interest included the numbers of fish caught, numbers of tagged fish caught, where the fish were caught, and the number of hours fished. Creel censusing was conducted daily for the first week after the two stocking dates. In addition, a weekly creel survey was conducted throughout the fishing season until 30 November (after which fishing pressure was minimal). Additional information was obtained through voluntary returns of fish tags by fishermen, telephone responses, and electrofishing surveys conducted in June and July. Nonparametric statistical procedures (Hollander and Wolfe 1973) were applied to the various data sets.

Results and Discussion

From a total of 1,061 marked trout, 326 tags (31%) were collected from anglers during the study (Table 1). Return rates for rainbow and brook trout were similar. The lower percentage of brown trout recaptured (16%) may have been related to their reduced catchability. Of these three species, brown trout are considered the most difficult species to catch (Cooper 1959). Of the 326 returns, anglers could supply information to adequately identify individual capture sites for 275 trout (85%). Information on capture sites for the remaining 15% of the returns was insufficient to evaluate dispersion. Recovery rates of tagged, hatchery-reared trout in other published studies have ranged from 5 to 17% (Bjornn and Mallet 1964), 33% (Cobb 1934), 13 to 25% (Moring 1980), 22 to 41% (Ratledge and Cornell 1953), and 1 to 38% (Shetter 1944). Our recovery rate was moderately high in comparison, and we believe our sample to be representative of the stocked population.

Table 1. Recovery rates of marked rainbow, brook, and brown trout stocked in Big Stony Creek, Virginia, May 1979.

Trout species	Number		Percent returned	Usable tags returned (No.)
	Tagged	Returned		
Rainbow	712	230	32.3	196
Brook	189	70	37.0	57
Brown	160	26	16.2	22
Total	1,061	326	30.7	275

Distance and Direction of Movements

In general, the stocked trout did not move far from their respective release sites (Fig. 1). Most (75%) were captured within 1 km of the release sites. A slightly greater proportion of stocked brook trout (19%), than brown trout (14%) or rainbow trout (12%), were recovered at the stocking sites.

Brook and rainbow trout moved primarily downstream, whereas brown trout moved upstream from initial release sites. Most brook trout (69%) and rainbow trout (59%) were recovered downstream from the release sites, but only a small proportion of the

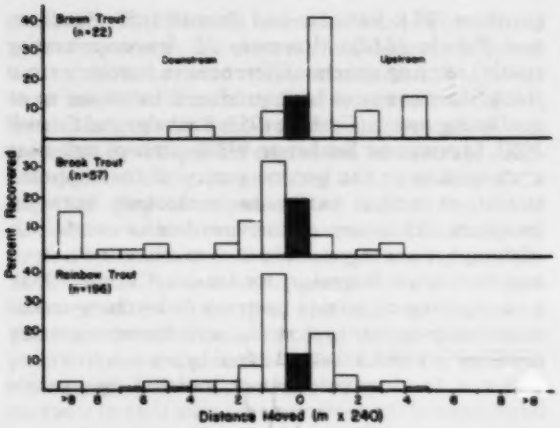

Fig. 1. Comparison of distance and direction of movement of hatchery-reared rainbow, brook, and brown trout stocked in Big Stony Creek (solid bars indicate the proportion of trout recovered within the 30-m release site).

brown trout (18%) were recovered downstream. In contrast, only 12% of the brook and 29% of the rainbow trout were captured upstream, whereas 69% of the brown trout were recovered upstream from the release sites.

The median dispersion distances for brook and rainbow trout were 195 and 60 m, respectively, downstream from the release sites, whereas for brown trout the median was 90 m upstream. The median dispersion distances for brook and rainbow trout were not significantly different from one another ($P = 0.05$, Dunn's [1964] multiple range test), but both were significantly different ($P < 0.001$, Kruskal-Wallis multiple range test) from that of brown trout. A few rainbow trout were recovered downstream beyond the study area (more than 3 km), but dispersion of most trout was generally restricted to short distances from the release sites.

A number of other researchers have also concluded that hatchery-reared trout are quite sedentary (Trembly 1943; Cooper 1952; Ratledge and Cornell 1953; Newell 1957; Bjornn and Mallet 1964; Bailey et al. 1973; Klein 1974). In most studies, dispersion of stocked trout averaged considerably less than 3 km. Movement studies of stream populations have indicated that most wild trout also remain in localized areas, often for long periods of time (Allen 1951; Miller 1957; Shetter 1968). Cargill (1980) found that wild rainbow trout (descendants of escaped hatchery fish) remained within a 165-m stream section for 2.5 years.

Although dispersion of trout stocked in Big Stony Creek was limited, relatively discrete directional movement patterns were observed among species. Both brook and rainbow trout exhibited a tendency to move downstream, whereas brown trout generally moved upstream from the release sites. Similar downstream movements following stocking have been reported for rainbow trout (Ratledge and Cornell 1953; Moring and Buchanan 1978), for brook trout (Cobb 1934; McCrimmon 1960), and for both species (Trembly 1943; Cooper 1952; Newell 1957). Upstream movements of stocked brown trout have been reported by Trembly (1943) and Brynildson (1967).

Effect of Habitat

To determine if the type of stream habitat (pools or riffles) into which the trout were released influenced patterns of dispersion, the data were partitioned by stocking habitat and analyzed accordingly. Regardless of habitat, most trout were recovered near their respective release sites. Of the 154 trout stocked into pools and the 121 trout stocked into riffles, 61 and 25%, respectively, were recovered within their initial release sites.

Although trout stocked into pools moved less than those stocked into riffles, the median dispersal distances of pool-stocked rainbow trout (60 m downstream), brook trout (30 m downstream), and brown trout (30 m upstream) versus those of riffle-stocked rainbow trout (60 m downstream), brook trout (210 m downstream), and brown trout (60 m upstream) were not significantly different (Wilcoxon rank sum test, $P = 0.757$, $P = 0.215$, $P = 0.447$, respectively). Brook trout stocked into riffles tended to move farther than those stocked into pools, but the dispersion ranges of brook trout stocked into these two stream habitats overlapped. This variability in dispersion tendencies combined with the relatively small sample sizes probably accounts for the absence of statistically significant differences in the movements of these two populations.

The type of stream habitat at the stocking site had no apparent effect on the directional movements of the three trout species. Rainbow and brook trout stocked in pools and riffles were recovered near or downstream from the release sites, whereas brown trout were generally recovered near or upstream from the release sites. Of those fish that moved beyond the initial 30-m release areas, most of the pool-stocked rainbow trout (65%) and brook trout (82%), as well as riffle-stocked rainbow trout (69%) and brook trout (89%), were recovered downstream from their release sites. In contrast, more pool-stocked brown trout (75%) and riffle-stocked brown trout (82%) were recovered upstream from their release sites. These relatively distinct species-specific directional movements were independent of stocking habitat.

Environmental Influences

Directional movements of stocked trout have been attributed to a variety of environmental factors, particularly water temperature and stream flow. Cooper (1952) found that although brook and rainbow

trout exhibited an immediate downstream movement when stocked at water temperatures of 10°C or less, they showed very little movement at warmer water temperatures; brown trout appeared to be unaffected by water temperature and generally remained in the vicinity of the stocking site. Newell (1957) also concluded that brook and rainbow trout tended to move downstream at water temperatures below 10°C. In the present study, downstream movements of brook and rainbow trout stocked in early May occurred at a mean water temperature of 12°C.

Some authors have speculated that increasing or decreasing streamflows may influence the directional movement patterns of trout. Brynildson (1967), Bailey et al. (1973), and Moring and Buchanan (1978) observed greater downstream movements of rainbow trout during high streamflows. Clothier (1953) reported a direct correlation between reduced flows and the upstream movement of brook, brown, and rainbow trout in an irrigation canal, but detected no such relationship in another canal in the same river system. Other investigators have concluded that flow rates had little or no effect on the movements of stocked trout (Newell 1957) or wild trout (Gerking 1953; Miller 1957). Downstream movements of brook and rainbow trout and upstream movements of brown trout occurred during normal spring flow conditions (3 m^3/s) in Big Stony Creek in the present study.

Additional Factors

Other factors, including the size and physical condition of the trout, mortality rates, interactions with wild trout, and habitat quality may have influenced the distributional patterns of stocked trout in Big Stony Creek. However, it is unlikely that any of these factors would differentially affect directional movements. Since all recaptured trout were harvested rapidly (90% of the returns were made within 10 days after stocking), the potential influences of variable mortality, competition, food supply, and habitat quality were probably minimal. The resident population of wild brown trout in Big Stony Creek was too small ($< 1/100$ m of stream length) to be considered a major influence in the dispersion of stocked trout. All three species, reared under the same hatchery conditions, were similar in size and vigor. Survival of these salmonids, held in the hatchery to evaluate tagging mortality, was identical (95%). It is equally unlikely that the observed movement patterns could be attributed to upstream spawning runs, since rainbow trout, the only spring-spawning species stocked, tended to move downstream.

Genetic variation between different strains of hatchery-reared salmonids, particularly rainbow trout stocks of suspected anadromous steelhead ancestry, has been suggested as a potential explanation to account for the movement patterns of stocked trout (Carbine 1953; Ratledge and Cornell 1953; Needham and Behnke 1962). However, the few comparative studies relating genetic differences in hatchery trout stocks to movement have produced inconclusive or conflicting results (Carbine 1953; Ratledge and Cornell 1953; Moring and Buchanan 1978), perhaps reflecting uncertainties in the genetic purity of the supposed strains, as well as variations in stocking methods, recapture techniques, and environmental conditions. Although it is recognized that any rainbow trout stock may have latent migratory tendencies (Carbine 1953), post-stocking dispersion patterns of hatchery-reared trout do not appear to represent an inherent migratory tendency similar to that exhibited by sea-run trout, but rather a localized movement initiated by specific conditions of the environment at the time of stocking (Cooper 1959). The trout used in this study were developed from eggs at the Coursey Springs State Fish Hatchery, Virginia. Pure strains of these stocks have not been maintained and the genetic characteristics of these fish have not been determined.

Although movement patterns of the unrecovered trout (69%) are not known, results of independent creel surveys (above and below the study area) as well as electrofishing surveys (within the study area) during June and July suggested that most of the unrecovered, tagged trout were harvested within the study area and not reported to the creel clerks. Very few tagged trout (less than 2% of the returns) were recovered outside the study area or within the study area 1 month after stocking. Observations by creel clerks indicated that during the period of heavy pressure (opening week), a number of anglers caught their limit and left the stream before being interviewed. A complete creel census was not conducted because of the high density of anglers (more than 50 anglers per kilometer). Moreover, reports of poaching and anglers exceeding their legal limit were not uncommon and undoubtedly accounted for some (moderate to large) proportion of the unrecovered tags.

The use of creel census to determine movement patterns involves certain unavoidable sociological limitations. For example, the validity of the movement data presented depends on obtaining accurate capture site information and assurance that angling effort was uniformly distributed throughout the study area. These were not, however, serious problems since most anglers were very cooperative in providing specific capture site information and equitably distributing themselves throughout the study area. Furthermore, all questionable data on capture site location (15% of the returns) were discarded.

Our results indicated that although the distances travelled by hatchery-reared rainbow, brook, and brown trout were generally restricted to within 1 km of the release site, directional dispersion tendencies of the species varied. Brook and rainbow trout moved

primarily downstream, whereas most brown trout moved upstream. Downstream movements of brook and rainbow trout and upstream movements of brown trout may have been related to environmental factors, particularly water temperature and streamflow, but may also have been an expression of inherited characteristics. Clearly, an increased understanding of the expected dispersion patterns of stocked trout and those factors which regulate movement is fundamental to the optimum use of hatchery resources and the success of catchable trout programs. For a comprehensive appraisal of the post-stocking movements of hatchery-reared trout, including the relevant European literature, the reader is directed to the excellent review provided by Cresswell (1981).

Acknowledgments

We thank Richard J. Neves, Carl B. Schreck, Diana L. Weigmann, and an anonymous reviewer for their thoughtful review and constructive criticism of the manuscript.

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Accepted 1 July 1980

Evaluation of Tilapia Meal for Fish Diets[1]

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ABSTRACT: Tilapia (*Tilapia mossambica*) were evaluated as a substitute fish meal for salmonid diets. Male and female tilapia of different sizes were analyzed for protein, lipid, ash, and gross energy content. Dry matter averaged 58% protein, and did not differ between sexes or sizes. Large (200 g) female tilapia had higher lipid and gross energy contents than did small females or males. Tilapia meal prepared from the entire body of small (100 g) male and female tilapia averaged 61% protein, 20% lipid, and 4.99 kcal/g of dry matter. Tilapia meal was substituted for herring meal in the Colorado standard diet. The growth and chemical composition of the carcass of rainbow trout (*Salmo gairdneri*) fed the two diets did not differ.

Periodic scarcity and high prices of herring and menhaden meals encourage the evaluation of alternative animal feed ingredients. *Tilapia* spp. are herbivorous and thus capable of converting inexpensive food substances into flesh (Bayne et al. 1976; Kirilenko and Mel'nikov 1969). *Tilapia* have a tendency to overpopulate, resulting in large numbers of small fish undesirable for human consumption (Chimits 1957). Fish from stunted populations may be ideally suited for use in commercial fish diets. Waste materials remaining after larger fish are filleted might also have potential as a feed ingredient.

Objectives of the present study were to examine differences in chemical composition between male and female tilapia (*Tilapia mossambica*) from two size categories. Biochemical composition of the entire body was also compared to waste remaining after fillets were removed from larger fish. Tilapia meal was substituted for herring meal in a conventional diet and the growth of fingerling rainbow trout (*Salmo gairdneri*) fed the two diets was compared.

Materials and Methods

Several phases of this study involved determination of moisture, crude protein, crude lipid, ash, and caloric content. Fish were prepared for analysis as follows. Fish were sacrificed, weighed to the nearest 0.1 g, and dried at 60°C until a constant weight was achieved. A Wiley mill was used to grind each fish individually to a fine consistency. The ground homogenates of each fish were placed in individual glass jars and stored in a desiccator. Replicate chemical analyses of each homogenate were performed. Samples of fish meals and final diets were ground in a Wiley mill and stored in desiccators until analysis was performed. Dried samples of fish homogenates, meals, and diets were analyzed as follows. Percentage ash was determined by combustion in a muffle furnace at 600°C for 4 h. Protein nitrogen was determined by the micro-Kjeldahl technique (Niederl and Niederl 1942) and converted to crude protein by multiplying by the factor 6.25. Gross energy values were determined with a Parr oxygen bomb calorimeter, and total lipid by reflux distillation with petroleum ether (Horwitz 1970).

Male and female tilapia were separated into two size categories (small ≅ 100 g live weight, large ≅ 200 g). Six individuals from each of the four groups were sacrificed and analyzed for moisture, protein, lipid, ash, and caloric content. In addition, eight large male tilapia were filleted and the chemical composition of the remaining carcass (i.e., offal) determined.

Suitability of tilapia meal as an alternative fish meal was evaluated by substituting it for herring meal in the Colorado standard diet, and feeding the standard and substituted diets (Table 1) to trout. Tilapia meal was prepared from about 35 kg of small tilapia. Fish were dried for 48 h at 60°C and ground in a Wiley mill; the resulting dry meal was homogenized. Fish meals were stored at 4°C until the diets were prepared. Diets of identical protein, lipid, and caloric content were desired, but differences in the chemical composition of

[1] Technical contribution 1897 of the South Carolina Agricultural Experiment Station, Clemson University.

Table 1. Formulation of experimental diets (percentage, except as otherwise indicated).

Ingredient	Colorado standard diet	Tilapia meal substituted diet
Fish meal (herring or tilapia)[a]	26.7	30.6
Wheat middlings[a]	17.9	17.4
Delactosed whey	10.0	10.0
Condensed fish solubles (dried)	10.0	10.0
Dried brewer's yeast	10.0	10.0
Corn fermentation extractives	6.5	6.5
Barley sprouts	6.5	6.5
Wheatgerm meal	5.0	5.0
Soluble dried blood flour	2.5	2.5
Stabilized fish oil (anchovy)[a]	3.4	0.0
Trace mineralized salt[b]	1.0	1.0
Vitamin premix[c]	0.5	0.5
Choline (g/10 kg)	2.2	2.2
Ascorbic acid (g/10 kg)	6.6	6.6

[a] Fish meal, wheat middlings, and fish oil were varied, depending on protein and lipid levels in the fish meal.

[b] Trace minerals in milligrams per kilogram of diet: zinc ($ZnSO_4$), 749; iron ($FeSO_4 \cdot 7H_2O$), 99; copper ($CuSO_4$), 31; manganese ($MnSO_4$), 749; iodine (KIO_3), 10.

[c] Vitamins in milligrams per kilogram of diet: thiamine mononitrate, 28; riboflavin, 28; pyridoxine HCl, 28; niacin, 385; d-calcium pantothenate, 83; folic acid, 17; d-biotin, 441; cyanocobalamin, 33; menadione sodium bisulfite, 9. Vitamins in international units per kilogram of diet: dl-α-tocopherol acetate, 220; A, 2,203; D_3, 441.

the two fish meals were anticipated. Thus, tilapia meal and herring meal were analyzed for crude protein, crude lipid, ash, and caloric content so that appropriate adjustments could be made during diet formulation. Each of the diets (Colorado standard and tilapia meal substituted) was mixed in an industrial mixer and pelletized into 3.2-mm pellets. Chemical analyses were performed on the final diets to verify similarity of composition.

Spring-hatched fingerling rainbow trout were obtained from the State of Colorado Division of Wildlife Experimental Hatchery and acclimated for 30 days in the laboratory at the University of Colorado. Fifty fingerlings were assigned randomly to each of six fiberglass troughs (1.0 × 0.3× 0.3 m). A semi-closed recirculating system was employed in which each trough drained into a common reservoir where water was thermostatically cooled and oxygenated, and dechlorinated water was added at a rate of 1 L/min. Water temperature was maintained at 11°C.

Three troughs, each containing 50 fish, were randomly assigned to each diet. All groups were fed twice daily, on the basis of a hatchery constant of 7.8 (Butterbaugh and Willoughby 1967). Rations were increased on a daily basis, on the assumption that growth would approximate 0.44 mm/day. Fish were weighed initially and at 2-week intervals for 12 weeks. Five groups of 10 fish were removed from each trough, placed in about 20 L of trough water, and weighed on a triple-beam balance. At the end of the 12-week period, 10 trout from each trough were killed and prepared for chemical analysis.

Analysis of variance was used to ascertain differences in proximate composition between large and small, male and female tilapia. Least-squares regressions were used to describe growth of rainbow trout, and the slopes for fish fed control and experimental diets were compared by analysis of covariance. Final chemical composition of rainbow trout was compared with nested analysis of variance.

Results and Discussion

Protein content of whole tilapia averaged 58% of dry weight, and did not differ between sexes or sizes (Table 2). Large female tilapia had significantly ($P < 0.05$) lower moisture and higher lipid content than males or small females. Lipid content of large female tilapia averaged 22.5% of the dry weight, compared with 16-19% for the other groups. Caloric density of large females averaged 5.26 kcal/g dry weight, whereas that of small females and males averaged between 4.34 and 4.81 kcal/g. Lipids ap-

Table 2. Analysis of tilapia evaluated for body size and sex differences in chemical composition. Each average[a] is followed by its standard error.

Fish	Live weight (g)	Moisture (%H_2O)	Carcass composition (% of dry matter)			Gross energy (kcal/g dry matter)
			Ash	Protein	Lipid	
Small						
Male	91.8 (5.5)	73.2 (1.7)w	23.2 (1.0)w	58.5 (1.4)w	19.4 (2.1)w	4.34 (0.15)w
Female	75.6 (3.0)	73.5 (0.2)w	22.8 (0.3)w	58.7 (0.7)w	15.8 (0.2)	4.68 (0.03)wx
Large						
Male	223.2 (7.5)	70.8 (1.2)w	23.1 (0.7)w	58.1 (0.7)w	17.2 (1.5)w	4.81 (0.10)x
Female	151.9 (11.0)	66.3 (3.6)	18.0 (0.5)	56.0 (1.0)w	22.5 (0.9)	5.26 (0.07)

[a] Values in a column followed by the same letter do not differ significantly at $P \leq 0.05$.

peared to be the primary constituent that differed between sizes or sexes; this difference is probably related to the reproductive cycle and ovarian development. Female tilapia used in the present study had not spawned. Unspent ovaries, known to have relatively high caloric content in other fishes (Foltz and Norden 1978), thus constituted a substantial percentage of dry body weight, thereby elevating the percentage of lipid and caloric values. Increased lipid content among larger individuals has been reported for the freshwater fish *Puntius gonionotus*, but whether the increase was sex-related was not determined (Tan 1971).

Fish meals vary considerably on the basis of their biochemical analysis and with regard to their nutritive properties (Hart et al. 1940; March et al. 1963). Variations are due to differences in types of fish used, parts of fish used (e.g., whole body minus fillet), season of capture, and processing methods (March et al. 1963). Generally, minimum acceptable protein values for fish meals ranged from 60 to 68% of the dry weight. Protein and lipid content of tilapia meal prepared in this study averaged 61 and 20% of dry matter, respectively, compared with 79% protein and 7% lipid for the commercially prepared herring meal. Tilapia meal, however, was prepared from the entire body, whereas commercial preparation of herring meals normally involves a defatting procedure. Commercial manufacture of tilapia meal would likewise require removal of most of the fat to improve storage characteristics.

Lipid removal would also be required for tilapia offal (whole body minus fillet). Eight large males produced fillets averaging 36% of the live weight. Analysis of resulting waste revealed 70% moisture, and a dry matter composition of 46% protein, 23% lipid, and 24% ash. Tan (1971) indicated that tilapia accumulate a relatively large amount of fat in muscle as opposed to the alimentary canal. In the present study, however, removal of the fillets resulted in an elevation of the lipid content by 6 percentage points and reduction in protein content by 14 percentage points, indicating that the muscular fillet is more protein-dense, whereas the remaining carcass is more lipid-dense.

Chemical analyses were performed on the Colorado standard and tilapia meal substituted diets to verify that both met projected compositions (Table 3). Ash content of the tilapia meal substituted diet averaged 15% of dry weight compared with 11% for the Colorado standard diet. Protein and lipid content of the substituted diet were 2 percentage points higher and lower, respectively, than the Colorado standard diet. Additional ash and a lesser amount of lipid in the substituted diet were responsible for a slightly lower gross energy content, which averaged 4.56 kcal/g in comparison with 4.68 kcal/g for the Colorado standard diet.

Growth and chemical compositions of two triplicate groups of rainbow trout did not differ, even though the Colorado standard diet had slightly higher caloric and protein densities. Analysis of covariance did not reveal significant differences ($P > 0.05$) of separate growth regressions. Final live weights averaged 39.0 and 36.1 g for rainbow trout fed the tilapia meal substituted and Colorado standard diets, respectively (Table 4). Percentage water was 79% of live weight for trout fed the tilapia meal diet and 77% for trout fed the Colorado standard diet. Carcass chemical composition for the two triplicate groups of rainbow trout did not differ ($P > 0.05$). Dry matter averaged about 68% protein, 19% lipid, 11% ash, and 5.45 kcal/g. Carcass protein and lipid values determined in this study were 4 percentage points higher and lower, respectively, than for rainbow trout fed the Colorado standard diet in a previous study (Windell et al. 1974). However, rainbow trout examined by Windell et al. (1974) were larger (e.g., 115 vs. 38 g) and had consumed a hatchery diet for a longer period. As the period of artificial rearing is increased, decreasing protein and increasing lipid levels characterize the carcasses of hatchery fish (Wood et al. 1957).

Table 3. Composition of fish meals and diets used in the rainbow trout growth experiment. Each average[a] is followed by its standard error.

Meal or diet	Moisture content (%H_2O)	Dry matter composition (%)			Gross energy (kcal/g dry matter)
		Ash	Protein	Lipid	
Tilapia meal	7.4 (0.1)	20.6 (0.1)	61.1 (0.5)	19.7 (0.3)	4.99 (0.02)
Herring meal	10.2 (0.1)	15.1 (0.1)	78.7 (0.7)	7.3 (0.4)	5.13 (0.01)
Tilapia meal substituted diet	9.7 (0.1)	13.1 (0.1)	40.1 (0.4)	9.6 (0.4)	4.56 (0.03)
Colorado standard diet	9.9 (0.1)	10.4 (0.1)	42.1 (0.4)	7.4 (0.5)	4.68 (0.02)

[a] Each average represents the mean of 12 determinations.

*Table 4. Effect of replacing herring meal with tilapia meal on the growth and body composition of fingerling rainbow trout (*Salmo gairdneri*). With the exception of water content, rainbow trout fed two different diets did not differ significantly ($P > 0.05$) in body size or proximate composition. Each average is followed by its standard error.*

Diet	Weight[a] (g)		Moisture	Carcass composition (% of dry matter)			Gross energy
	Wet	Dry	(% H_2O)	Ash	Protein	Lipid	(kcal/g)
Tilapia meal substituted	39.0 (1.5)	8.41 (0.35)	78.5 (0.2)	11.5 (0.1)	69.0 (0.8)	17.9 (0.8)	5.44 (0.03)
Colorado standard	36.1 (1.8)	8.16 (0.40)	77.4 (0.2)	10.7 (0.1)	67.1 (0.7)	19.5 (0.9)	5.46 (0.03)

[a] Average final weight after 12 weeks for rainbow trout having an initial body weight of 23.0 g.

We conclude that tilapia are a suitable source of fish meal for fish diets. Future studies should investigate the economic feasibility of large-scale production of fish meal from tilapia cultured in thermal effluents or eutrophied waters.

Acknowledgments

We thank Larry Harris of the Colorado Division of Wildlife for technical assistance in pelleting the experimental feed and supplying rainbow trout, Burly Jenkins for supplying tilapia, and Dylan Gibson for valuable laboratory assistance.

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Accepted 1 September 1981

Furunculosis in Brook Trout: Infection by Contact Exposure

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ABSTRACT: In juvenile brook trout (*Salvelinus fontinalis*) challenged by applying about 10^9 virulent *Aeromonas salmonicida* cells to an abraded area along the lateral line, or held in aquarium water containing about 10^5 cells per milliliter, mortalities were 50 and 60%, respectively, within 7 days after exposure. Neither feeding the bacterium to the fish nor applying it directly to the gills caused systemic bacterial infection. Two consistent experimental challenges were developed for inducing furunculosis in juvenile brook trout: (1) 15-min contact (bath) exposures of trout to about 10^6 *A. salmonicida* cells per milliliter of spring water, and (2) 60-s dip exposures of the fish to 10^9 *A. salmonicida* cells per milliliter. These challenge procedures produced 70-100% and 74-88% mortality, respectively, within 14 days after exposure. Results obtained with a strain of brook trout from Owhi Lake, Washington, by the dip exposure method were affected by neither the age of the fish (up to 1 year) nor the source of the population. The challenge was also effective for Atlantic salmon (*Salmo salar*) but not for rainbow trout (*S. gairdneri*).

The development of a bacterin for control of furunculosis is complicated by the lack of challenge procedures that consistently induce experimental infections that can be used to provide an accurate assessment of the protection conferred to fish by a bacterin. *Aeromonas salmonicida* infections have been induced experimentally by injection of the pathogen (Michel 1980), by horizontal transmission (Bullock et al. 1976), or by waterborne exposure (Spence et al. 1965), but results have been inconsistent. Others (Spence et al. 1965; Udey and Fryer 1978) exposed fish to natural outbreaks of furunculosis, but the use of such an exposure method necessitates proximity to an enzootic area. The present study was undertaken to develop a practical method for the consistent induction of furunculosis in brook trout (*Salvelinus fontinalis*).

Preliminary Infectivity Trials

The *Aeromonas salmonicida* isolate used in the present experiments was isolated from diseased brook trout at the Leetown (West Virginia) National Fish Hatchery in September 1978. Tryptic soy broth (TSB) was inoculated with this isolate and incubated 24 h at 20°C. Cultures were adjusted with 0.85% saline to 5% transmittance at 525 nm on a Spectronic 20 colorimeter and quantified by the drop-plate technique (Miles and Misra 1938).

Groups of 10 juvenile brook trout (average weight and fork length, 17.5 g and 104 mm) were maintained in 40-L aquaria receiving 1.5 L/min of 12.5°C spring water and exposed to one of four types of challenge: (1) Fish were fed 10 g of food coated with 5.0 mL of culture. (2) Fish were anesthetized (0.4 mg/L tricaine methanesulfonate) and placed on a dampened cloth; 0.5 mL of culture was then pipetted onto the gills. The fish were held for 30 s before being returned to the aquarium. (3) Fish were anesthetized and placed on a dampened cloth as in challenge 2. Scales along the left lateral line area were then abraded with a Pasteur pipette, the abraded areas were covered with 0.5 mL of culture, and the fish were held for 30 s before being returned to the aquarium. (4) In a bath exposure, 5 mL of culture was poured directly into the aquarium. Water flow was stopped for 30 min and then restored. A control group of 10 fish was held under comparable conditions but not challenged.

Mortalities in each group were recorded for 7 days and the presence of an *A. salmonicida* infection was confirmed by the direct fluorescent antibody technique (FAT) of Bullock et al. (1980). Kidney smears from survivors were examined by FAT on the 8th day to determine the incidence of infection.

Only one mortality and a single incidence of infection was observed among survivors of fish offered contaminated food. The application of 10^9 bacterial cells to the gill surface did not result in transmission of the disease (Table 1). High mortalities and infective incidences were, however, observed among fish challenged either by application of 10^9 cells to abraded scales along the lateral line or by additions of 2.5×10^5 cells/mL to the water.

*Table 1. A comparison of routes of inoculation for infecting juvenile brook trout (*Salvelinus fontinalis*) with* Aeromonas salmonicida.

Method of inoculation	Challenge dose (cells/mL)	Mortality in 7 days[a] (%)	Infection among survivors	
			Survivors (No.)	Infected (%)
In feed	2.0×10^9	10	9	11
Abrasion	2.0×10^9	50	5	40
Waterborne	2.5×10^5	60	4	80

[a] Ten fish per group.

Development of Experimental Challenges

In view of the successful induction of disease by either skin abrasion or bath exposure, and because of greater ease of application by bath exposures, waterborne transmission of the bacterium was the mode selected for further experiments. In the first experiment, trout were exposed to dense suspensions (10^6 cells/mL of water) of *A. salmonicida* in the challenge vessel. The *A. salmonicida* isolate was inoculated onto tryptic soy agar (TSA) slants and incubated for 24 h at 20°C. Bacterial growth was washed from each slant with 3.0 mL of TSB, and 2.0 mL of the resulting suspension were used to inoculate 200 mL of TSB. These cultures were incubated at room temperature (20-22°C) for 48 h on a rotary shaker at 200 rpm. This culture was diluted in 4.8 L of 12.5°C spring water and aerated. Ten juvenile brook trout were placed in this suspension for 15 min and then returned to 40-L aquaria. Five groups of brook trout were challenged, each with a fresh culture. Mortalities were recorded daily for 14 days and infections were confirmed by direct FAT.

Furunculosis was successfully transmitted in each of the five groups of brook trout exposed. The first death was observed 4 days after exposure and mortalities ranged between 70 and 100% within 14 days. Mortalities did not occur among fish exposed to sterile broth culture. The mortality among the five challenged groups averaged 88 ± 12%.

For the second experiment, additional cultures were grown in 7.0-mL brain-heart infusion (BHI) broth, incubated for 24 h at 20°C, and 5.0 mL of the resulting suspension were used to inoculate 500 mL of BHI broth. Three cultures prepared in this manner were placed on a rotary shaker at 200 rpm and incubated for 48 h at room temperature. The cultures were combined aseptically and plate counts were determined (Miles and Misra 1938).

In a dip exposure challenge, groups of 25 brook trout were dipped for 60 s in 1.5 L of a challenge culture containing about 1.3×10^9 cells per milliliter. Challenged trout were maintained in groups of 50 fish in six 40-L aquaria. Mortalities were recorded daily for 14 days and infections were confirmed by direct FAT.

Challenge of juvenile brook trout by the 60-s dip exposures produced furunculosis in each of the six groups. First mortalities were observed 4 days after challenge and major losses occurred from 6 to 9 days after exposure. No mortality occurred in controls. The average mortality for the six challenged groups was 82 ± 8%.

Because it was easier to dip fish for 60 s in a culture containing 10^9 *A. salmonicida* cells per milliliter than it was to challenge them in a sustained water bath, dip exposure was examined to determine the effects of challenge on different life stages and populations of fishes. To determine if susceptibility to the challenge changed with the age of the fish, triplicate groups of 25 brook trout of the Owhi Lake (Washington) strain, obtained from White Sulphur Springs (West Virginia) National Fish Hatchery, were challenged at ages of 3, 6, and 12 months. Fish from another population of 3-month-old Owhi-strain brook trout, obtained from the Paint Bank (Virginia) National Fish Hatchery, were challenged to determine if brook trout from different sources responded uniformly to challenge. Three-month-old Atlantic salmon (*Salmo salar*) and Wytheville-strain rainbow trout (*S. gairdneri*) were also challenged. Each dip exposure challenge was conducted with cultures ranging from 2.0 to 6.0×10^9 cells per milliliter of *A. salmonicida* to provide additional data on the reproducibility of the procedure. Appropriate unchallenged groups served as controls.

The results of these studies indicated that the dip exposure challenge procedure is reproducible, that Owhi-strain brook trout less than 1 year old responded similarly to the challenge, and that there was not a significant difference in challenge results from two different populations of Owhi-strain brook trout (Table 2). However, within 9 days after challenge all Atlantic salmon were dead, whereas none of the challenged rainbow trout had died up to 1 month after exposure.

Discussion

Although bacterial contact on abraded skin produced infections and mortality in brook trout, abrasion was inconvenient, required individual handling, and proved to be an unnecessary step when compared with the results of a bath challenge with 2.5×10^5 cells per milliliter in water. In the definitive experiments, both bath exposure for 15 min to 10^6 *A. salmonicida* cells per milliliter of challenge suspension, and 60-s dip exposure in 10^9 cells per milliliter provided practical means of consistently inducing

Table 2. Cumulative mortalities 14 days after dip exposure of fishes in 10^9 cells/mL of Aeromonas salmonicida.

Species	Source NFH[a]	Age (months)	Number of deaths per challenge[b] Group 1	Group 2	Group 3	Average mortality (%)
Brook trout (Owhi strain)	White Sulphur Springs	3	20	23	22	88
		6	23	21	18	83
		12	20	21	18	80
Brook trout (Owhi strain)	Paint Bank	3	21	19	19	80
Atlantic salmon	Cortland	3	25	25	25	100
Rainbow trout (Wytheville strain)	White Sulphur Springs	3	0	0	0	0

[a] NFH = National Fish Hatchery.
[b] Each group contained 25 fish.

furunculosis. Because the dip exposure method is effective in inducing furunculosis and is easier to apply, the dip procedure is proposed as a practical challenge method for consistently producing diseased brook trout.

Neither age nor source of the brook trout populations used in this study altered challenge results. Although the procedure reproducibly produced furunculosis in Atlantic salmon, the challenge dose of 10^9 cells per milliliter was apparently too high for this species. Further studies with graded bacterial concentrations are needed to establish a standardized challenge for this species. The procedure was not effective in producing furunculosis in rainbow trout and this is attributed to the greater resistance of this species than either brook trout or Atlantic salmon to furunculosis. Use of the dip exposure technique on susceptible fishes (i.e., brook trout and Atlantic salmon) should facilitate determination of the efficacy of chemotherapeutants and vaccines.

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Accepted 29 July 1981

Inhibition of Spermatogenesis and Vitellogenesis in Rainbow Trout by Hormonal Additives in the Diet

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ABSTRACT: In 1976, groups of rainbow trout (*Salmo gairdneri*) that were prepuberal males and adult females undergoing their second reproductive cycle were given methyltestosterone (MT) or estradiol (E) in pelleted feed at dosages of 50 or 500 μg/kg of feed (groups of fish received each dosage of each hormone) during the period of spermatogenesis and full vitellogenesis between 17 May and 21 November. This treatment completely inhibited gametogenesis. Although vitellogenesis did not occur in the females, no major changes were observed in the ovaries, which remained at the previtellogenetic stage with apparently normal oocytes and oogonia. Drastic changes in the male testes were found, however, with disappearance of spermatogonia at the highest doses of MT and E. In sexually mature fish, MT did not appear to be a growth promoter. In both males and females, E inhibited growth and induced high mortality.

In fish farming, it is most economical to raise animals without gonads or in which the gonads remain undeveloped. When this method is used, growth of the fish is not retarded by the onset of sexual maturity and the feed conversion ratio is better (Simpson 1976); there is no evisceration loss owing to the presence of gonads (such as that found in males of intermediate size and in large females of various salmonid species produced in fresh water or in sea cages); and the taste and appearance of sterile fish is not altered by sexual maturation.

Within certain limits, prepuberal salmonids may be produced by manipulating the photoperiod to retard the onset of puberty (Bourlier 1979), by irradiating embryos (Tashiro 1972), or by polyploidy (Allen and Stanley 1978; for a review, see Chevassus et al. 1979). Fish may also be sterilized by hormonal treatment with sex steroids during sexual differentiation; sterile fish, sexual inversions, and hermaphrodites have thus been obtained (Jalabert et al. 1975; Yamazaki 1976; Johnstone et al. 1978; Goetz et al. 1979).

The potential value of treating adult animals with hormones has been little studied. We know only that anabolizing treatments cause total or partial gonadal (especially testicular) regression (Hirose and Hibiya 1968; Fagerlund and McBride 1975; Higgs et al. 1977; Fagerlund et al. 1979). The results of such treatment are, however, species-dependent and the treatment does not always inhibit gametogenesis (McBride and Fagerlund 1973, 1976; Fagerlund and McBride 1977; Fagerlund et al. 1979).

We present the results of some sterilization experiments on male and female rainbow trout (*Salmo gairdneri*) in which methyltestosterone (MT) and estradiol-17β (E) were incorporated into the feed during gametogenesis.

Materials and Methods

Male and female rainbow trout, originating from local private fish farms, were brought to the laboratory on 10 May 1976 and held under natural photoperiod in recycled water (Petit 1974) in our experimental facilities at a temperature of 14–17°C. Spermatogenesis and vitellogenesis had not started and the gonads were in the resting state. The males were prepuberal and the females were beginning their second reproductive cycle. A week later, all the fish were tagged individually with a mouth bar and weighed; they were divided into 5 groups, each containing 10 to 15 fish of both sexes. The fish were fed a pellet diet supplemented with MT at 50 μg (group 2) or 500 μg (group 3) per kilogram of feed or with E at 50 μg (group 4) or 500 μg (group 5). The steroids were dissolved in oil and included in the pellets. Control fish (group 1) received the pellet diet without steroids. Feed was given once a day, 6 days per

week, at a daily rate of 1% of body weight. In June the temperature of the water rose to 20–22°C and the feeding rate was reduced to 0.5%.

On 19 November, when gametogenesis was completed, the steroid treatment was withdrawn and the fish were given the pelleted feed without steroid additives until the end of the experiment (10 January 1977). During this period, the daily mean water temperature fluctuated between 8 and 20°C. Body weight was periodically measured during the experiment; at the end of the experiment, the gonads, liver, and viscera (excluding the liver) were weighed to enable calculation of the gonadosomatic index (GSI), hepatosomatic index (HSI), and viscerosomatic index (VSI). A sample of flesh from the dorsal musculature anterior to the dorsal fin was stored at −20°C until radio-immuno-assay (RIA) for steroid residues could be performed.

After the tissues were ground in a homogenizer, they were hydrolyzed briefly (10 min) with alkali (2 mL KOH 60% + 20 mL distilled water). The estradiol was then extracted by dichloromethane and the testosterone by cyclohexane-ethylacetate (50/50) to eliminate any lipids that might interfere with the RIA. The serum against testosterone, at a dilution of 1:12,000, crossed 100% with the testosterone, 77% with androstane 5α -β diol, 16.7% with dihydroxytestosterone, 0.33% with androstanedione, 1.43% with dehydroepiandrosterone, and 0.2% with epitestosterone. The serum against estradiol, at a dilution of 1:16,000, was specific for estradiol-17β, and only crossed 7% with estrone and 0.9% with estradiol-17β. Samples were incubated for 3 h at 4°C and the free estrogen was separated from the antibody-bound estrogen by adding 1 mL of dextran charcoal (250 mg of active charcoal and 25 mg dextran for 100 mL of phosphate buffer). The results were statistically compared by analysis of variance after log transformation of the data for comparison of the GSI values.

Results

The steroid treatment entirely inhibited vitellogenesis in females, and the GSI was always significantly lower in the treated fish than in the controls ($P < 0.001$; Table 1). The oocytes were at the previtellogenetic stage, but oogonia and meiotic oocytes were still present in the ovaries in all groups. The steroid treatment significantly reduced the GSI in males ($P < 0.001$). In groups 3, 4, and 5, spermatogenesis was totally inhibited and no germ cells were visible in most testes. Spermatogenesis was not completely inhibited and germ cells were still present in fish of group 2, which had received the smaller dose of MT.

Under steroid treatment, the males did not adapt well to their new environment at the beginning of the experiment and lost weight (Fig. 1). The controls and MT-treated fish resumed growth, but E-treated fish continued to lose weight until day 140, when they started to grow. At the end of the experiment, their final weight was still lower than their initial weight, but the differences were not significant (Table 1). In females, only the treated fish, and especially the E-treated ones, lost weight significantly ($P < 0.05$; Fig. 2, Table 1). In both males and females, mortality in the groups treated with E was much greater than in the other groups. The VSI was not significantly changed by the treatment, but E-treated fish tended to have a

Table 1: Some mean effects (SD in parentheses) of methyltestosterone (MT) and estradiol-17 β (E) given in pelleted feed to rainbow trout males and females from 17 May 1976 to 10 January 1977.

Sex group and treatment	Steroid dose (μg/kg feed)	No. of fish		Mean weight (g)		Indices[a]			Residues	
		Initial	Final	Initial	Final	GSI	HSI	VSI	Androgens	Estrogens
Males										
1 Control	0	13	12	223 (21)	231 (47)	2.58 (0.11)	2.22 (0.39)	5.37 (0.8)	0.58 (0.2)	0.84[b] (0.51)
2 MT	50	15	14	236 (26)	241 (40)	0.79[d] (0.5)	3.75 (1.57)	3.17 (1.5)		
3 MT	500	11	8	233 (16)	231 (30)	0.46[d] (1.4)	1.98 (0.82)	5.90 (0.8)	0.50[c] (0.84)	
4 E	50	10	4	227 (40)	218 (42)	0.39[d] (0.6)	3.60 (0.11)	6.60 (0.6)		0.17 (0.1)
5 E	500	10	1	236 (25)	201	0.25	3.10	5.60		
Females										
1 Control	0	11	8	789 (29)	854 (112)	20.0[c] (1.6)	2.59 (0.3)	5.02 (0.84)	0.33 (0.17)	1.27 (0.24)
2 MT	50	10	8	766 (49)	825 (175)	0.76[d] (0.5)	2.24 (0.2)	4.96 (0.51)		
3 MT	500	10	8	839 (38)	792 (167)	0.26[d] (0.1)	1.97 (0.13)	4.95 (0.24)	0.21 (0.22)	
4 E	50	10	6	957 (158)	761[e] (117)	0.87[d] (0.3)	2.50 (0.4)	4.40 (0.61)		0.64 (0.13)
5 E	500	10	3	901 (148)	662[e] (107)	0.68[d] (0.3)	4.30 (0.96)	5.50 (0.82)		1.1

[a] The indices are the gonadosomatic (GSI), the hepatosomatic (HSI), and the viscerosomatic (VSI).
[b] One outlying value was 2.16 mg/g.
[c] Most of the control females had ovulated; gonadal weight includes remnants of ovaries plus weight of ovulated eggs.
[d] $P < 0.001$ (comparison with control).
[e] $P < 0.05$ (comparison with initial weight).

higher HSI (Table 1). The residues found in these fish 45 days after withdrawal of the treatment were similar to those in the control fish (Table 1).

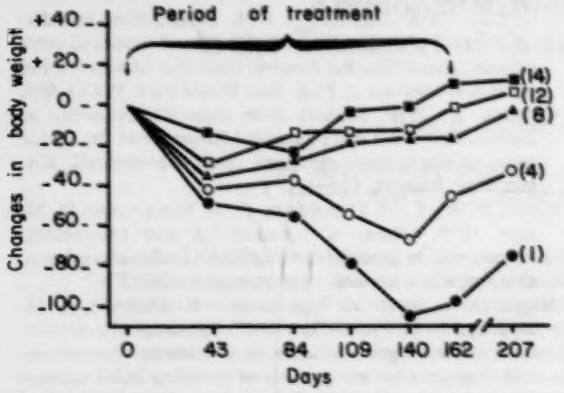

Fig. 1. Average cumulative change in body weight of male rainbow trout during the experimental administration of methyltestosterone (MT) and estradiol (E) in pelleted feed. Dosages, in micrograms per kilogram of food, were administered as follows: Group 1 (□ Control), none; Group 2 (■), 50 MT; Group 3 (Δ), 500 MT; Group 4 (○), 50 E; Group 5 (●), 500 E. The numer of fish surviving at the end of the experiment is given in parentheses.

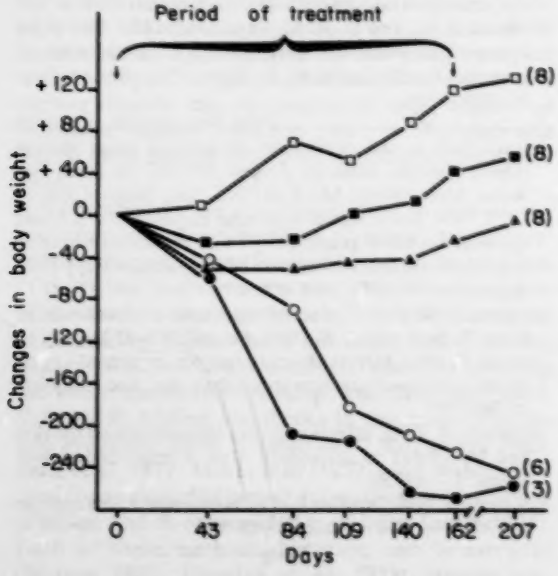

Fig. 2. Average cumulative change in body weight of adult female rainbow trout during the experimental administration of methyltestosterone (MT) and estradiol (E) in pelleted feed. Dosages for groups of females were the same as those for males (shown in Fig. 1). The number of fish surviving at the end of the experiment is given in parentheses.

Discussion

Results of the present study show that methyltestosterone and estradiol, when added to the feed given at the time of maximal gonadal activity, can inhibit gametogenesis in the rainbow trout. However, since E induced high mortality and inhibited growth, it would be of no practical use. Although MT had no effect on survival and growth in males, it affected growth in females slightly, especially at the larger dosage. It appears, therefore, that MT may be used as an antifertility compound. This result is of interest for the production of pan-sized fish, in which the proportion of mature males is relatively high.

The growth-promoting effect of MT on juvenile rainbow trout reported by Fagerlund and McBride (1977) was not found in the present experiment with prepuberal males receiving as much as 500 μg/kg of feed. Since only a limited number of fish were involved, however, our conclusions cannot be considered definitive. Simpson (1976) stated that MT was less effective in promoting growth in rainbow trout than in the genus *Oncorhynchus*. In the present study, growth of adult females was inhibited by MT in comparison with that of the controls. To our knowledge, this result has not been reported before because most experiments demonstrating the growth-promoting effect of androgens have involved immature underyearling or yearling salmonids.

Estradiol definitely retarded the growth of male and female fish, as previously reported (Ashby 1957). Ghittino (1970) observed that diethylstilbestrol was ineffective as a growth promoter in rainbow trout; the feed with steroid added may not have been well accepted by the fish, leading to starvation and sterility. It is known, however, that feed-restricted trout will produce eggs when fed at a daily rate as low as 0.4% of body weight (Billard and De Fremont 1980). The poor growth generally observed even in the controls at the beginning of the experiment may have had any of several origins: handling and tagging the fish, transfer in recycled water, or exceptionally high rearing temperature in June with a lower feeding rate.

Histologically the ovaries were unmodified by treatment with MT or E. Oogenesis was stopped at an early previtellogenetic stage, as noted by Yamazaki (1972); therefore, we believe the effect of the treatment is probably reversible. Changes in males were much more significant, with complete sterility and total disappearance of the germ cells observed after dosages of MT (500 μg/kg) and of E (at both dosages). At the smaller dose of MT (50 μg/kg), some spermatogenetic activity was observed in the testes in which some resumption of mitotic activity had occurred after treatment was discontinued.

Residues of androgens and estrogens in the muscle 45 days after treatment was discontinued were less

than 0.5–0.6 ng/g; these findings agree with those of other studies (Fagerlund and McBride 1978; Fagerlund and Dye 1979), but the possibility that various hormonal metabolites may persist remains undetermined. The residues of androgens and estrogen found in untreated, mature fish were similar to those found in treated fish.

Acknowledgments

Methyltestosterone and estradiol were provided by the pharmaceutical firm ROUSSEL Laboratories, Paris. We acknowledge the assistance of Alice Daifuku for the English presentation of the manuscript.

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Accepted 21 July 1981

Ammonia Removal by Clinoptilolite in the Transport of Ornamental Freshwater Fishes[1]

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ABSTRACT: The addition of the zeolite mineral clinoptilolite to polyethylene shipping bags containing goldfish (*Carassius auratus*) substantially reduced the concentration of ammonia in the water during 24 h of simulated transport, in comparison with controls without clinoptilolite. At stocking densities of about 22 or 44 g of fish per liter of water, 10, 20, and 40 g/L of clinoptilolite consistently decreased the concentration of total ammonia-N by about 73, 87, and 93%, respectively. Concentrations of un-ionized ammonia-N never exceeded 0.017 mg/L in bags containing even the lowest weight of clinoptilolite, whereas concentrations as high as 0.074 mg/L were found in control bags without clinoptilolite.

The major factors limiting the transport of fishes in sealed containers are changes in temperature, depletion of dissolved oxygen, increases in acidity and carbon dioxide, and accumulation of toxic nitrogenous wastes, notably ammonia. Changes in temperature can be moderated by the use of insulated shipping boxes; adequate concentrations of dissolved oxygen can be maintained by displacing the air above the transport water with pure oxygen; and increases in acidity and carbon dioxide can be controlled with organic or inorganic buffers (Nemoto 1957; McFarland and Norris 1958; Braker 1974; National Research Council 1974).

Two methods are commonly used to control the accumulation of ammonia in transport water: preventing ammonia formation by altering the metabolism of the fishes, and removing ammonia from the water after it has been excreted. Some recommended methods of decreasing the metabolic rate (and, hence, the rate of ammonia excretion of confined fishes) are lowering the water temperature (Phillips and Brockway 1954; Voss 1979), adding anesthetics to the water (Phillips and Brockway 1954; Nemoto 1957; McFarland 1960; Smit et al. 1977; Murai et al. 1979), and withholding food for an extended period before the fish are shipped (Phillips and Brockway 1954; van den Sande 1974). Each of these methods, however, can be stressful (Buxton 1962; Houston et al. 1971; Strange and Schreck 1978; Ishioka 1980) and, when exacerbated by the unavoidable stress of handling and confinement, they may contribute to high shipping mortalities. Umminger and Gist (1973) and Strange et al. (1977), for example, have shown that fishes are more easily stressed by handling as their acclimation temperature is lowered. A variety of ion-exchange resins have the ability to remove ammonia from fresh water. Saha et al.(1956) found synthetic Amberlite resins to be effective, and Nemoto (1957) reported that a cation-exchange resin (type unspecified) kept ammonia at low levels in aquaria and in shipping bags.

Clinoptilolite is a naturally occurring zeolite with a typical unit-cell formula of $Na_4K_4Al_8Si_{40}O_{96}\cdot 24\ H_2O$. Although it has a high selectivity for ammonium ions in the presence of competing cations such as Mg^{2+}, Ca^{2+}, and Na^{+} in fresh water (Mercer and Ames 1978), it is ineffective in seawater (Johnson and Sieburth 1974). The theoretical equilibrium exchange capacity of clinoptilolite for ammonium is 2.25 meq/g; reported equilibrium capacity values, however, range from about 1.2 to 2.25 meq/g (Koon and Kaufman 1971; Breck 1974; Sims and Hindin 1978; Klieve and Semmens 1980), and depend on factors such as the purity of the clinoptilolite ore, the type and degree to which it is preactivated, the exact exchange-cation composition of the clinoptilolite, and the composition of the water being treated. Clinoptilolite has been used successfully for the removal of ammonia from waste water and agricultural runoff (Breck 1974; Murphy et al. 1976; Jorgensen et al. 1976; Mercer and Ames 1978), and its potential as an alternative to bacteriological filtration in closed-system aquaculture also has been demonstrated (Johnson and Sieburth 1974). We therefore conducted a study to determine the effectiveness of clinoptilolite for controlling the accumulation of ammonia in sealed shipping bags during the simulated transport of ornamental freshwater fishes.

[1] Contribution No. 28, Sea Research Foundation, Inc.

Materials and Methods

Clinoptilolite (−4 × 14 U.S. mesh) from a deposit in Buckhorn, New Mexico, was provided by the Double Eagle Petroleum and Mining Company, Casper, Wyoming. The clinoptilolite was crushed and graded to grain sizes of 2–5 mm (about 4 × 12 U.S. mesh), rinsed several times with distilled water to remove the fines, and dried at 200°C for 1 h. At the beginning of each experimental trial, the clinoptilolite was weighed and then rinsed with distilled water before being distributed to the shipping bags. The water used in the simulated shipping trials was dechlorinated tap water that had been recirculated through granular activated carbon for 24 h. The mean values for water quality at the start of the three trials were as follows: temperature, 20.8 ± 1.6°C; pH, 7.72 ± 0.16; dissolved oxygen, 8.3 ± 0.6 mg/L; total ammonia-N, 0.039 ± 0.003 mg/L; and un-ionized ammonia-N, 0.001 ± 0 mg/L. Hardness and alkalinity were not measured; however, for the tap water used in the study, total hardness averaged 16–24 mg/L and total alkalinity 7–11 mg/L (data provided by the Metropolitan District, Hartford, Connecticut).

The experimental animals were goldfish (*Carassius auratus*), an extremely hardy species with a high tolerance for ammonia (Wilson et al. 1969). Four groups each of 10 and 20 fish (average weight per fish, 1.1 g) were transferred to polyethylene bags 15.2 × 40.6 cm, 0.038 mm thick. The bags contained 500 mL of water and 0, 5, 10, or 20 g of clinoptilolite. The stocking densities were about 22 and 44 g/L in bags containing 10 and 20 fish, respectively. The bags were flattened to the water surface to expel the air, and then inflated with bottled oxygen gas and sealed with elastic bands; the resulting volumetric ratio of oxygen to water was 5:1. Each sealed bag was placed inside another polyethylene bag to ensure against water loss from perforation or leakage, and the bags were transferred to Styrofoam boxes. After 24 h had elapsed, the bags were opened and water samples were collected and analyzed immediately for dissolved oxygen, pH, and total ammonia-N.

Concentrations of dissolved oxygen were measured with a model OX-2P test kit (Hach Chemical Co., Ames, Iowa). According to Boyd (1980), values obtained with this test kit are within 96% of those obtained by standard analytical methods. Determinations of pH were carried out with a model 501 pH meter (Orion Research Inc., Cambridge, Massachusetts). Total ammonia-N (NH_4-N) was determined potentiometrically with an Orion model 407 specific-ion meter and an Orion model 95-10 ammonia electrode. Values for un-ionized ammonia-N (NH_3-N) were calculated from the equations given by Emerson et al. (1975). The statistical significance of differences between water quality in the control bags and in bags containing clinoptilolite was determined with Student's *t*-test.

Results and Discussion

The presence of 5, 10, and 20 g of clinoptilolite in the shipping bags (10, 20, and 40 g/L, respectively) reduced the concentrations of NH_4-N by about 73, 87, and 93%, respectively, as compared with values in the control bags (Table 1). The percentage of ammonia removed by a given weight of clinoptilolite was independent of the concentration of ammonia; however, the weight of ammonia removed by a given weight of clinoptilolite was directly proportional to the concentration of ammonia. These observations are consistent with the findings of Jorgensen et al. (1976, 1979) and with the Langmuir adsorption theory, as modified by the Freundlich equation (Barrow 1966:766). Concentrations of dissolved oxygen in all the shipping bags were above saturation values, and were not significantly different in bags with and without clinoptilolite ($P > 0.10$). The decreases in pH after 24 h were smaller in bags containing clinoptilolite than in the controls. This effect was most pronounced in shipping bags containing the lowest weights of fish and the greatest weights of clinoptilolite. Williams (1974) observed that in water from a fish-rearing tank the pH increased by about one unit after passage through a column of clinoptilolite, but that no increase in alkalinity was noted. The elevation of pH by clinoptilolite probably results from the removal of hydrogen ions from the water by exchange with sodium and potassium ions (Breck 1974: 529–592; Jorgensen et al. 1976).

Un-ionized ammonia generally is regarded as the form that is toxic to aquatic animals; the proportion of NH_3 in aqueous solution depends mainly on temperature and pH, tending to increase as the temperature and pH of the solution increase. In the present study the transport water was not buffered; consequently, all pH values of the water after 24 h were below 7.0, and un-ionized ammonia-N represented less than 0.5% of total ammonia-N. Concentrations of NH_3-N as high as 0.074 mg/L were found in the control bags, whereas values never exceeded 0.017 mg/L in bags containing even the least clinoptilolite.

Although most species of freshwater fishes can tolerate short-term exposures to concentrations of NH_3-N below 0.15 mg/L (European Inland Fisheries Advisory Commission 1973), 24-h TL_m values as low as 0.052 and 0.075 mg NH_3-N/L have been reported for rainbow trout (*Salmo gairdneri*) fry and adults, respectively (Rice and Stokes 1975). Apparently the toxicity of ammonia to ornamental species of freshwater fishes has not been studied. It should be borne in mind, however, that the concentrations of NH_3-N in the shipping bags without clinoptilolite equaled or

Table 1. Mean[a] values (with standard deviations in parentheses) for water quality characteristics in shipping bags containing 500 mL water, 10 or 20 goldfish, and 0, 5, 10, or 20 g of clinoptilolite after 24 h of simulated transport.

No. fish per gram of clinoptilolite	Total weight of fish (g)	Temp. (°C)	pH	Dissolved oxygen (mg/L)	NH_4-N (mg/L)	NH_3-N (mg/L)	NH_4-N removed[b]	
							Percent	Milligrams per gram of clinoptilolite
10/0 (Control)	10.7 (0.7)	23.4 (0.4)	6.65 (0.10)	15.0 (1.7)	9.97 (1.90)	0.023 (0.007)	—	—
10/5	10.5 (0.6)	23.4 (0.3)	6.77 (0.13)	15.7 (2.5)	2.73[c] (0.19)	0.008[d] (0.002)	72 (6)	0.723 (0.188)
10/10	10.6 (0.6)	23.2 (0)	6.83[d] (0.11)	16.3 (1.2)	1.24[c] (0.30)	0.004[c] (0.002)	88 (1)	0.437 (0.081)
10/20	11.0 (0.6)	23.3 (0.1)	6.93[d] (0.07)	16.3 (0.6)	0.708[c] (0.233)	0.003[c] (0.001)	93 (2)	0.238 (0.044)
20/0[e] (Control)	21.3 (0.4)	23.6 (0.5)	6.60 (0.14)	11.0 (2.6)	21.2 (3.6)	0.048 (0.023)	—	—
20/5	21.0 (0.3)	23.4 (0.4)	6.64 (0.09)	12.3 (0.6)	5.65[c] (1.29)	0.013[d] (0.005)	73 (4)	1.56 (0.27)
20/10[e]	21.3 (0.5)	23.4 (0.3)	6.69 (0.06)	11.7 (1.2)	2.96[c] (0.82)	0.007[d] (0.002)	86 (3)	0.912 (0.157)
20/20[f]	21.7 (0.4)	23.4 (0.2)	6.75 (0.06)	13.3 (1.2)	1.38[c] (0.25)	0.004[d] (0.001)	93 (2)	0.495 (0.086)

[a] Mean of three replicate trials.
[b] Compared to control without clinoptilolite.
[c] Significantly different from the control at the 0.01 level of probability.
[d] Significantly different from the control at the 0.05 level of probability.
[e] One of 60 fish dead after 24 h.
[f] Two of 60 fish dead after 24 h.

exceeded concentrations that may be lethal to species that are highly susceptible to ammonia intoxication.

If the water in the present study had been buffered to counteract the accumulation of respiratory carbon dioxide, as recommended by McFarland and Norris (1958), the potential for toxic effects from un-ionized ammonia would have increased dramatically. Concentrations of NH_3-N, calculated from the data in Table 1 at a theoretical pH of 8.0, are given in Table 2. At this pH value, the mean concentrations of NH_3-N in the control bags containing 10 and 20 fish without clinoptilolite would have been 0.48 and 1.04 mg/L, respectively, falling well above threshold concentrations of un-ionized ammonia that are toxic in short-term tests with several species of freshwater fishes (European Inland Fisheries Advisory Commission 1973). In bags containing even the greatest weights of fish and the lowest weights of clinoptilolite, however, the concentration of NH_3-N would have been below the 24-h LC50 values for most species.

The present study demonstrated that clinoptilolite is effective for removing ammonia from fresh water during the transport of fishes in sealed containers. Ammonia removal by ion exchange is preferable to methods that reduce the rate of ammonia excretion (water cooling, anesthetization, and starvation), because it is not stressful. Although small particles of clinoptilolite remove ammonia more effectively than

Table 2. Theoretical concentrations[a] of un-ionized ammonia-N (NH_3-N) in transport water buffered to pH 8.0.

No. fish per gram of clinoptilolite	NH_3-N (mg/L)
0/0[b]	0.002
10/0 (Control)	0.480
10/5	0.132
10/10	0.059
10/20	0.034
20/0 (Control)	1.04
20/5	0.272
20/10	0.143
20/20	0.066

[a] Calculations based on temperatures and concentrations of NH_4-N from Table 1, using the equations of Emerson et al. (1975).
[b] Based on pre-experimental water quality.

large grains because of their greater surface area (Jorgensen et al. 1976), large grain sizes are desirable for shipping purposes because they do not cause the water to become turbid. Extensive deposits of clinoptilolite are located throughout the western United States; clinoptilolite is available directly from mining companies for about $60 to $150 per metric ton. Small quantities of clinoptilolite may be purchased from distributors of aquarium supplies at considerably higher cost.

Data from the present study suggest that the weight of grains of Buckhorn clinoptilolite (2 × 5 mm) should be no less than one-half the weight of the fish, to maintain acceptably low concentrations of un-ionized ammonia during transport in sealed bags. Although there are no published reports comparing the survival of fish transported with and without clinoptilolite, it seems reasonable that the removal of ammonia from shipping water by ion exchange, when other methods are used concomitantly to maintain constant temperature, low concentrations of carbon dioxide, and adequate concentrations of dissolved oxygen, should greatly reduce transport mortalities of ornamental freshwater fishes.

Acknowledgments

We thank J.B. Gratzek, P. W. Johnson, J. F. Kuhns, and F. A. Mumpton for criticizing the manuscript and for their helpful discussions about ion exchange by zeolites. Bottled oxygen gas was provided by the Aero All-Gas Company, Hartford, Connecticut.

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Accepted 8 September 1981

Range Pellets as Supplemental Crayfish Feed

A major problem in crayfish farming is depletion of vegetation as a feed (Avault et al. 1974). In the absence of vegetation, crayfish stunt at submarketable sizes (< 75 mm total length). Hays and agricultural by-products are potential supplemental feeds (Romaire et al. 1976; Goyert and Avault 1977; Rivas et al. 1978). Romaire et al. (1978) concluded that stunted crayfish could be induced to grow with supplemental feeding. Rivas et al. (1978) found that adding agricultural by-products to crayfish ponds increased production significantly, although the high cost of labor and hauling did not justify their use at a commercial level. Use of high protein pelleted rations as supplemental feed to increase crayfish production has been investigated (Smitherman et al. 1967; Meyers et al. 1970), and Clark et al. (1974) reported that crayfish grew well on pellets, although high production costs limited their use. Reduction of agricultural by-products to pelleted rations makes application easier and more economical by permitting a more even distribution of the materials into ponds. Use of a low-cost pelleted ration formulated for cattle from agricultural by-products (range pellets) may have potential as a crayfish food supplement, but information is lacking.

Sixteen rectangular 0.014-ha ponds at Louisiana State University's Ben Hur Farm in Baton Rouge were used for our study; 12 were planted with rice (*Oryza sativa*) at a rate of 153 kg of seed per hectare in June 1979, and four were not planted. In late September all ponds were flooded with well water to an average depth of 60 cm. Crayfish were not stocked because natural populations of *Procambarus clarkii* and *P. acutus acutus* were already present.

Sixteen ponds were randomly assigned to one of four treatments. In treatment one, four ponds without planted vegetation received a pelleted ration from 25 September 1979 to 3 May 1980; in treatment two, four ponds planted to rice received the pelleted ration from 25 September to 3 May and, in treatment three, four rice-planted ponds received the pelleted ration only from 1 March through 3 May. Four rice-planted ponds received no supplemental feed and served as a control.

The supplemental feed, a pelleted livestock ration called "range pellets," consisted of cottonseed meal and elevator grain screenings with a molasses binder. Crude protein content of the pellets was 9.0%, minimum crude fat content was 2.0%, and maximum crude fiber content was 12.0%. Average duration of pellet stability in water was 22 min (N=20). Low cost ($90/ton; 1979) and easy availability appeared to make these pellets attractive as a supplemental feed for crayfish. Pellets were broadcast by hand into ponds twice a week at a rate of 59 kg/ha through February. Feed was not distributed on days when water temperature dropped below 13°C. Rates were increased on 1 March to 98 kg/ha per feeding, and maintained at that level until the study ended on 3 May.

Water temperature and dissolved oxygen were measured daily in all ponds, and total alkalinity and pH were measured monthly. Water quality variables were within desirable ranges for crayfish production, although blue-green algae caused minor water quality problems in April in all ponds receiving pellets.

Crayfish were harvested daily from 12 February through 3 May, using two upright small-mesh (6-mm)

and two upright large-mesh (20-mm) traps baited with gizzard shad (*Dorosoma cepedianum*) in each pond.

Young-of-the-year crayfish (5 to 10 mm total length) were present from September through April, indicating that reproduction occurred throughout the study. The high levee:water ratio of the ponds provided a large area for burrowing and subsequent reproduction, which led to overcrowding and increased aggression.

A highly significant difference ($P < 0.01$) in crayfish yields existed between the four treatments (Table 1). Treatment 2, with rice-planted ponds and supplemental pellets from September through May, produced the highest average yield of crayfish, with 2,130 kg/ha. Treatment 3, with rice-planted ponds and pellets added for the last 2 months of the study, yielded 2,016 kg/ha. The rice-only treatment (control) produced an average 1,274 kg/ha, and the pellets-only treatment (treatment 1) yielded 881 kg/ha.

Table 1. Crayfish production in ponds planted with rice or with no vegetation, with and without supplemental range pellets.

Pond treatment	Average yield[a] (kg/ha)	Standard deviation (kg/ha)	Marketable crayfish[b] (%)
Pellets only	881x	107.5	5.2
Rice planted only	1,274y	250.3	35.7
Rice planted and pellets (March–May)	2,016z	154.73	38.7
Rice planted and pellets (September–May)	2,130z	425.68	48.0

[a] Means followed by the same letter are not significantly different; the differences between means followed by different letters are highly significant ($P < 0.01$).
[b] Total length, > 75 mm.

Although treatment 2, with supplemental range pellets in rice-planted ponds, increased the total crayfish yield above that attained in the control ponds (rice alone), it did not prevent stunting of crayfish. Severe stunting was evident in all ponds by late February. Ponds without vegetation produced stunted crayfish in late January despite the supplemental feed they received, suggesting that feed availability was of little importance in the absence of sufficient vegetative substrate. During the early months of the study, thick stands of rice provided substrate and forage for the expanding crayfish population. As rice decayed, the carrying capacity of planted ponds decreased, despite the addtion of supplemental feed. Lack of forage led to increased aggressive interaction among crayfish and ultimately had a negative effect on growth. A forage crop that provides adequate substrate throughout the entire growing season is needed to maximize crayfish production.

Acknowledgment

Pellets used in this study were obtained through the courtesy of Louisiana Premium Livestock Feeds, St. Francisville, Louisiana.

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Accepted 29 July 1981

Manuring Rates for Production of Blue Tilapia in Simulated Sewage Lagoons Receiving Laying Hen Waste

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ABSTRACT: All-male blue tilapia (*Tilapia aurea*) were stocked at 50 fish per pool in 7-m² plastic wading pools which received direct input of laying hen manure at the rates of 0, 35, 70, or 140 kg dry weight per hectare per day during 1978 and 70, 140, 210, or 280 kg dry weight per hectare per day during 1979. On the basis of growth and survival of blue tilapia and of water quality data we suggest that the manuring rate in summer should be between 70 and 140 kg per hectare per day, depending on the alkalinity of the water.

The accumulation of manure in poultry houses poses a problem for the poultry industry because of objectionable odors and the production of flies. One method of eliminating manure buildup and the resulting odor and fly problems would be to construct poultry houses with concrete floors under the cages and have a water-flush system carry the manure to an outdoor sewage lagoon. After settling of the manure the water could be recycled through the flush system.

Sewage lagoons provide a convenient method of treating waste water by biological and physical action in a pond or series of ponds. High organic loading in such lagoons usually stimulates dense phytoplankton blooms, which can be used by phytophagous fish.

Fish are produced in many parts of the world in sewage lagoons or bodies of water fertilized by animal manures (Bardach et al. 1972; Brown 1977; Buck 1977). *Tilapia* spp. are excellent candidates for use in such systems because of their demonstrated tolerance of high levels of ammonia and low levels of dissolved oxygen (DO), and their largely herbivorous food habits (Balarin and Hatton 1979).

As much as 15% of commercial poultry diets consist of costly fish meal. The tilapia produced in poultry sewage lagoons can be processed into fish meal and fed to poultry at levels up to 10% of the diet without producing off-flavors in either eggs or meat (Rowland et al. 1978). If such a system is to be economically feasible, however, the value of the fish produced must offset the construction and maintenance costs of the system and also offset the value of the manure in some alternate use, such as fertilizer for field crops. Since fish meal is inexpensive relative to the cost of fish for human consumption, the latter use might also be an economical alternative in a poultry-tilapia operation.

The present study was designed to determine the number of laying hens and loading rate of manure per hectare of sewage lagoon required to achieve maximum growth of blue tilapia (*Tilapia aurea*) at a fixed fish-stocking density. The effect of different experimental manuring rates on water quality and resulting production and growth by simulated sewage lagoons were examined during 1978 and 1979.

Materials and Methods

The present study was conducted at the Aquaculture Research Center of the Texas Agricultural Experiment Station near College Station, Texas, in 12 outdoor circular plastic-lined wading pools, 3.0 m in diameter and 0.6 m deep, each containing about 4,200 L of water. Pool bottoms were covered to a depth of about 10 cm with washed builder's sand. Four airstones were spaced equidistantly around the perimeter in each pool to provide water circulation simulating wind-generated circulation in an actual sewage lagoon.

Direct manure input was provided by suspending cages containing laying hens directly over the pools on wooden frameworks. The cages were protected from rain and direct afternoon sunlight with galvanized sheet metal roofs. This study used 1.6-kg (average) white leghorn laying hens, 1.5 years old, to provide a relatively constant manuring rate (on a per bird basis) throughout the experiment. The hens were fed ad libitum (112 ± 10 g per hen per day) with a commercial corn-soybean ration for laying hens.

The experiments had four treatment levels of laying hens, with three replicates of each treatment. Treatment levels in 1978 were 0, 1, 2, and 4 hens per pool, which is the equivalent of 0, 1,380, 2,760, and 5,520 hens/ha (0, 35, 70, and 140 kg of dry manure per hectare per day). In 1979, the treatments were 2, 4, 6, and 8 hens per pool, which is the equivalent of 2,760, 5,520, 8,380, and 11,040 hens/ha (70, 140, 210, and 280 kg of dry manure per hectare per day).

In 1978 the pools were initially filled with sand-filtered water from a 4-ha storage reservoir with an

alkalinity of about 200 mg/L as $CaCO_3$. In 1979, the pools were initially filled with water from a deep well with an alkalinity of about 800 mg/L as $CaCO_3$. Reservoir water was added as needed during both years to replace evaporative losses. In addition, during each experiment about 20 L of pond water were added to each pool to encourage development of phytoplankton and bacteria.

Each pool was stocked with 50 juvenile male blue tilapia (average, 1.0 g per fish; 61,425 fish per hectare). All-male fish were produced by feeding tilapia fry a trout starter ration containing 60 mg/kg of 17α-ethynyltestosterone for 3 weeks before stocking (Guerrero 1975).

Betwen 0800 and 0900 h daily, temperature was measured and DO determined with an oxygen meter; pH and total ammonia nitrogen were determined by using specific-ion electrodes. Orthophosphate, nitrite, nitrate, total hardness, total alkalinity, and hydrogen sulfide were measured weekly (American Public Health Association et al. 1975). Un-ionized ammonia concentrations were calculated from temperature, pH, and total ammonia nitrogen data by the method of Hampson (1977). Water quality mesurements were discontinued if all the fish in a pool died.

Computer analysis of variance and Duncan's multiple range test were performed by using the Statistical Analysis System, SAS-79 (Barr et al. 1979), to delineate statistically significant differences in measured factors as a function of treatment. All statistical tests were evaluated at the 0.05 level. Correlation coefficients and their associated *P*-values were determined among all measured water quality factors.

Results and Discussion

Growth and Survival of Fish

Tilapia growth in 1978, as determined from final mean weights (Table 1), was significantly higher in the treatments with 2 and 4 hens than those with 0 or 1 hen. Growth in the treatments with 2 and 4 hens was not significantly different, indicating that at the highest manuring rate the fish were either unable to make efficient use of the available nutrients or, more probably, that water quality limitations prevented those fish from growing at a rate more rapid than that of fish exposed to the second highest manuring rate.

Water quality impairment did not appear to be sufficient to cause mortalities in 1978. Survival ranged from 92 to 100%, with no significant differences among treatments. We concluded that under the 1978 experimental conditions the fish could tolerate a stocking rate of 2,760 to 5,520 hens/ha (70 to 140 kg of dry manure per hectare per day).

No fish survived in the treatments with 4, 6, and 8 hens in 1979 because of extremely poor water quality. Survival in the treatment with 2 hens per pool ranged from 88 to 98% and mean final weight was 44.9 g/fish. Comparison of tilapia growth in the treatments with 2 hens in 1978 and 1979 revealed no significant difference in final mean weights.

Shell (1966) reported that growth rates of fish in plastic pools with a substratum are greatly inferior to growth rates of fish in ponds. Tilapia growth rates during 1978 and 1979 were poor in comparison with results from previous manuring studies in ponds at the same facility (Burns and Stickney 1980). Although the absolute growth rates from this study cannot be extrapolated to production in actual sewage lagoons, the relative growth rates demonstrated for the treatment levels used in this study nevertheless help to delineate optimum manuring rates.

Water Quality

Average morning water temperatures were 25.9 ± 0.5°C (N=86) in 1978 and 25.4 ± 0.5°C (N=109) in 1979. The average morning temperature for 20 weekly measurements in 0.05-ha ponds at the Aquaculture Research Center in 1979 was 27.1°C (Rouse 1981). Metabolic rate and, therefore, fish growth rate, in tilapia increases with water temperature up to about 28 to 30°C (Balarin and Hatton 1979). Thus, lower pool temperatures may have contributed to reduced growth in pools in comparison with results previously obtained under similar conditions in ponds (Burns and Stickney 1980).

Average morning concentrations of DO decreased considerably as the manuring rates increased (Table 2). During the 1978 experiment DO did not appear to adversely affect tilapia growth or survival. Growth was greatest in the treatment with 4 hens per pool. Levels of DO in 1979 were substantially lower on the average than in 1978. Low levels of DO in the

Table 1. Mean final weight and percent survival[a] of blue tilapia in simulated sewage lagoons, 1978 and 1979.

Treatment		1978		1979	
Hens per pool (no.)	Dry weight of manure (kg/ha per day)	Weight (g)	Survival (%)	Weight (g)	Survival (%)
0	0	3.6x	94x		
1	35	29.2y	100x		
2	70	42.8z	100x	44.9	92
4	140	46.5z	97x	—	0
6	210			—	0
8	280			—	0

[a] Values in each column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 2. Treatment means[a] of the most significant water quality factors for the production of blue tilapia in simulated sewage lagoons to which laying hen wastes were added, 1978 and 1979.

Treatment		Dissolved oxygen (mg/L)		Un-ionized ammonia (mg/L)		Alkalinity (mg/L as $CaCO_3$)	
Hens per pool	Equivalent dry manure (kg/ha per day)	1978	1979	1978	1979	1978	1979
0	0	7.8x		0.001x		196x	
1	35	6.9y		0.025x		253xy	
2	70	6.1y	4.1x	0.04 xy	0.285x	290y	860x
4	140	5.0z	2.6y	0.077y	0.520y	357z	918x
6	210		2.8y		0.690z		997x
8	280		1.9y		0.727z		955x

[a] Values in each column followed by the same letter are not significantly different ($P \leq 0.05$).

treatments with 4, 6, and 8 hens in 1979 may have acted synergistically with high levels of ammonia to kill the fish. Such synergisms should be studied further to delineate the relations among various water quality factors and toxicity.

Levels of un-ionized ammonia (Table 2) increased significantly as the manuring rate increased. When the treatment levels (hens per pool) were the same, concentrations of un-ionized ammonia were much higher in 1979 than in 1978. The elevated levels of ammonia in 1979 apparently resulted from the higher alkalinity in the pools in that experiment.

Bouldin et al. (1974) have shown that significant amounts of ammonia are lost from ponds by volatilization to the atmosphere when pH exceeds 8.5. This also appears to be a major mechanism for ammonia loss from the simulated sewage lagoons in this study. To some degree this loss may have offset the relatively high toxicity of ammonia at high pH (which is associated with the known percentage increase of un-ionized ammonia with increasing pH). When carbon dioxide is removed through photosynthesis pH is elevated and the buffering capacity of the water is reduced. With moderate alkalinities during 1978, photosynthetic activity led to diurnal pH fluctuations of as much as 1.0 pH unit, which may have led to volatilization of ammonia during the afternoon. Conversely, in 1979 the high buffering capacity (as indicated by high alkalinity) limited pH fluctuations to 0.1 unit daily and lower pH levels were maintained. Volatilization of ammonia was retarded and nitrification apparently was not sufficiently rapid to prevent the accumulation of ammonia to lethal levels in 1979.

A significant negative correlation was found between morning pH levels and ammonia concentrations in all manured pools during both years. This finding substantiates the theory that in 1978 ammonia was being lost from the system through volatilization when pH was elevated in the afternoon. At that time ammonia was concentrated only in pools which experienced a phytoplankton population crash, which greatly reduced the rate of photosynthesis and consequently eliminated the large diurnal fluctuation in pH.

Levels of hydrogen sulfide, nitrite, and nitrate generally remained low and had no apparent effect on tilapia growth or survival in either year. Orthophosphate levels increased significantly with increasing manuring rate.

Conclusions

Optimum manuring rate for tilapia production in a simple sewage lagoon may be expected to vary along a broad continuum, depending on water chemistry and climatological factors. The present study with simulated sewage lagoons demonstrated that blue tilapia can be reared in central Texas during the summer with laying hen waste inputs from 70 to 140 kg dry weight per hectare per day. It is suggested that the lower end of the range should be used in water of high alkalinity and the higher end may be safe in waters of low alkalinity. Further experimentation in pilot-scale facilities are needed to refine the manuring rate in this 70- to 140-kg range before projections on a commercial scale can be made.

Volatilization of ammonia from water of low alkalinity may be an important mechanism of removal in systems of the type described here, even though high pH leads to increased ammonia toxicity. The apparent ability of tilapia to adapt to high ammonia may be a factor (Redner and Stickney 1979).

Acknowledgments

This research was supported, in part, by the Texas Agricultural Experiment Station project H-2831. We thank W. A. Isbell for assistance in facilities construction, and Lenton O. Rowland, Department of Poultry Science, Texas A&M University, who provided laying hens and feed.

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Accepted 20 July 1981

Common Carp Eggs as a Starter Diet for the Intensive Culture of Smallmouth Bass Fry

The intensive culture of largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieui*) has been a major point of interest to fish culturists in recent years. Culturists would prefer to culture fry soon after swim-up; however, most have found it necessary to begin with bass of nearly 50 mm initial length to successfully train fish to feed on a prepared diet. Snow (1960, 1968) found this to be true for largemouth bass, as did Puttmann (1973) for smallmouth bass. Anderson (1974) developed a training diet sequence for smallmouth bass in which Oregon moist pellet (Hublou 1963) gradually replaced ground flesh of common carp (*Cyprinus carpio*). Although Anderson (1974) found this method was most successful for fish 50–70 mm long, he also trained fish as small as 25 mm. An attempt to train 12-mm largemouth bass fry by this method was not successful (Murphy 1980). Brandenburg et al. (1979) used eggs of the common carp as a food source for largemouth bass 20 mm long (range 16–26 mm). We found that common carp eggs were a useful diet for largemouth bass fry as small as 10 mm and that fry which had been trained to eggs readily switched to Biodiet (Bioproducts, Inc.), a moist prepared diet (Willis and Flickinger 1981).

The objective of the present research was to determine whether common carp eggs were a suitable starter diet for intensively rearing smallmouth bass fry of various initial sizes.

The test conditions we used were those that had proven most successful in our trials with largemouth bass fry (Willis and Flickinger 1981). Smallmouth bass fry were reared in covered red plastic buckets having a standpipe drain system and a water capacity of 10 L. Water temperature was held at 22°C by heating well water. A 24-h continuous photoperiod was provided by overhead fluorescent light banks. Density of fish in the rearing buckets was 10 fish per liter (100 fish per bucket). Fish were fed five times per day at 3-h intervals, beginning at 0700 h. An overabundance of food was provided, which necessitated daily cleaning of rearing containers. Flow into the buckets was about 1 L/min. Training success was defined as the ratio of fish present at the conclusion of the trial (minus any

visually emaciated non-feeders) to those present at the start, multiplied by 100 to obtain a percentage.

Two trials were set up — one to determine training success and another to determine whether smallmouth bass trained to accept common carp eggs would later switch to a prepared diet. For the training success trial, smallmouth bass fry of initial lengths from 10 to 20 mm were collected and reared for 9 days on eggs. The 20-day switching trial was replicated twice with 20-mm fry under the previously described test conditions and involved the following training sequence: 7 days common carp eggs; 1 day 75% eggs, 25% Biodiet; 1 day 50% eggs, 50% Biodiet; 1 day 25% eggs, 75% Biodiet; and 10 days Biodiet alone. Fish for all trials were collected from College Lake, Colorado, with the exception of the 10-mm fry, which were collected from a private pond in LaPorte, Colorado. All fish were collected between 13 and 25 June 1980. Trials were conducted in the wet laboratory in the Natural Resources Building on the Colorado State University campus.

Training success of smallmouth bass fry was 45% for 10-mm fry but increased to 76% for 12-mm fry and reached a peak of 86% for 20-mm fry (Table 1). The difference in training success between 10- and 12-mm fry was significant (chi-square test, 1 df, $P < 0.005$) but no significant difference between 12- and 20-mm fry ($P > 0.1$) was observed. The 10-mm initial length would appear to border on the minimal size of fish that can accept common carp eggs. Culturists might increase training percentages substantially by allowing fry to remain in spawning ponds for a few more days until a slightly larger size (12 to 15 mm) was reached. The switching trial showed that smallmouth bass fry trained to accept common carp eggs would switch to Biodiet. The 20-mm fry had training successes of 82 and 85% and grew from 20 to 31 mm during this trial.

Table 1. Training success and growth of smallmouth bass fry of various initial lengths fed common carp eggs for 9 days.

Starting date (1981)	Source of fish	Training success (%)	Length (mm)	
			Initial	Final
25 June	Flickinger pond	45	10	13
13 June	College Lake	76	12	18
13 June	College Lake	79	12	18
13 June	College Lake	82	14	20
20 June	College Lake	80	18	23
20 June	College Lake	86	20	25

We had noted a problem with scoliosis in largemouth bass fry fed common carp eggs, apparently because of tryptophan deficiency (Willis and Flickinger 1981). Scoliosis was more limited in the smallmouth bass fry fed on eggs — only 4 to 9% incidence in fry 10–14 mm long, and none in bass 18–20 mm long.

Common carp eggs proved to be a useful starter diet for smallmouth bass fry and trained fry readily switched to a moist, prepared diet. However, a large-scale trial should be conducted to see whether results would be the same in large rearing containers. We would like to point out that in a similar experiment largemouth bass fry performed as well in a large-scale trial as they did in experimental tests (Willis and Flickinger 1981).

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Accepted 8 September 1981

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Central System for Monitoring the Quality of Water from Many Sources

Research under way at Sunndalsøra required frequent measurement of dissolved oxygen concentration (DOC) in the water from the outlets of 33 fish tanks and also from the water supply source. A monitoring system (Fig. 1) was therefore developed in which (a) multiple water samples (up to 35) flow by gravity from the various sources to the sampler through 10-mm clear polyvinyl chloride (PVC) tubing; (b) a water sampler diverts one water sample at a time to the measuring equipment; (c) sampled water passes over an oxygen probe; (d) a meter displays the DOC; (e) reference information from photocells in the water sampler is transmitted to a data compiler; (f) the data compiler coordinates information from the water sampler and the oxygen meter; (g) a data logger records reference information and DOC on magnetic tape; and (h) a pen recorder displays DOC at each source over the previous few hours. Items e, f, and g are needed only when a permanent record (or data analysis) is required. Although the system described operates on gravity flow, water samples can, if necessary, be delivered to the system by a multichannel peristaltic pump.

At Sunndalsøra this system is part of an experiment designed to estimate genetic factors in food conversion efficiency and growth in rainbow trout (*Salmo gairdneri*). The system is used to help arrive at estimates of food consumption in 33 tanks, each containing a full sib family of fish. For each tank, oxygen consumption is calculated over a period for which both the flow rate (Kinghorn 1981) and the mean drop in DOC between inlet (fresh) and outlet (used) water are known. An estimate of total energy metabolized, derived from the oxygen consumption (Brett and Gross 1979), is added to the energy component of growth, to give a value for total energy assimilated. When the energy digestibility and the energy component in the diet are known, the energy and food consumed can be calculated.

A wider application of the system is that of monitoring oxygen levels in fish tanks, as a security measure. An alarm system can be connected to the oxygen meter output and programmed to respond to a predetermined level of DOC. Use of this single-probe system for monitoring dissolved oxygen in several tanks avoids the costs of purchase and maintenance of multiple oxygen probes. The use of different types of probes and meters enables the monitoring of one or more other properties of water, such as pH, salinity, ammonia concentration, sulfur dioxide concentration, and suspended matter (by light absorption).

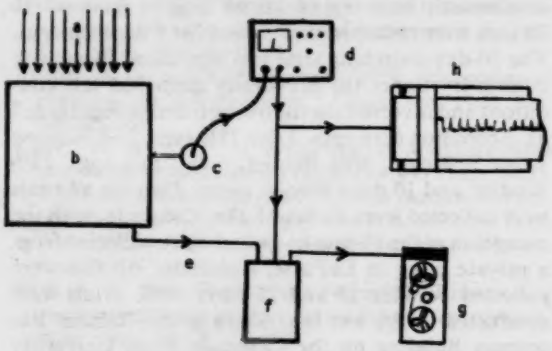

Fig. 1. Schematic diagram of water sampling system (arrows indicate the direction of information flow): (a) incoming samples from tank outlets and water supply; (b) water sampler; (c) oxygen probe; (d) dissolved oxygen meter; (e) reference information from photocells; (f) data compiler; (g) data logger and magnetic tape; (h) pen recorder for visual monitoring of the system.

The water sampler is a box constructed of perspex (Figs. 2, 3) with 35 input tubes, of which 33 carry water from the fish tank outlets and 2 carry water from the water supply source. One output tube carries a sample from one source at a time to the oxygen probe and the other carries the water from all other sources to the waste outlet. Each input tube is connected at the top of the sampler (Level I, Fig. 2) to a Y junction (*3*) with one end open. Excess pressure is released by overflow (*2*) at this open end; the overflow is regulated by a restrictor (*9*) adjusted so that the water flow rate over the oxygen probe (*11*) is equal for each of the sampler's 35 channels. At any time, flow from one channel is diverted at an intermediate level (Level II) by the pickup arm (*7*) and led to the oxygen probe (*11*) by means of a swivel joint (*20*); water from all other channels drops freely from Level II to the bottom level (Level III) and flows to the waste drainpipe (*15*). The pickup arm (*7*) is mounted on a ratchet cog wheel (*8*) that is activated once every minute to move the pickup arm to the next channel.

A photocell (Fig. 2, *18*) is mounted at the 1-rpm electric motor (*17*), which drives the ratchet mechanism (see Fig. 3) and activates the recording equipment just as the pickup arm moves to the next channel. The measuring equipment is allowed 1 min to equilibrate at each channel before a reading is taken. A slower motor

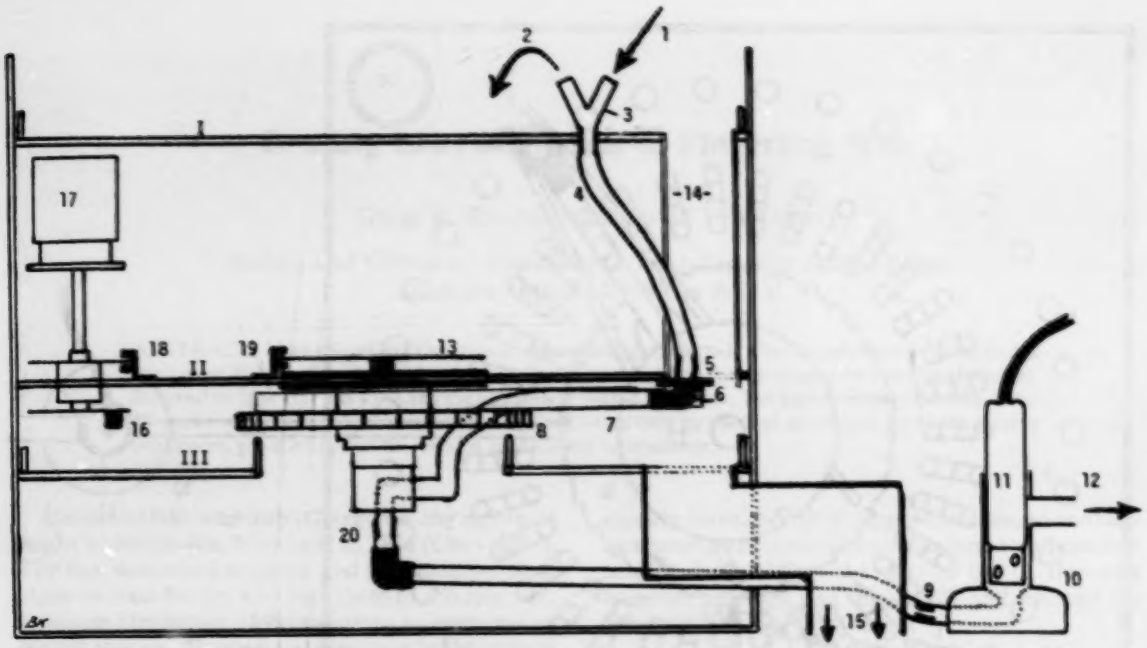

Fig. 2. Side view of water sampler (arrows indicate direction of water flow). The three gravity flow levels are designated I (top level, incoming samples), II (intermediate level), and III (bottom level). 1, sample water input, through 10-mm tubing (only one of the 35 channels is shown); 2, overflow due to back pressure from restrictor (9); 3, Y junction (one per channel); 4, connection tube (one per channel); 5, brass connector, where water is either diverted for sampling or directed to level III for waste discharge; 6, collection cup, spring-equipped to form a seal against the perspex sheet of Level II so that leakage is negligible; 7, pickup arm; 8, 35-tooth ratchet wheel on which the pickup arm is mounted (ratchet mechanism is detailed in Fig. 3); 9, restrictor, adjusted so that the channel with the lowest input pressure just overflows at the open end of its Y junction, ensuring the same flow rate over the oxygen probe for water from each channel; 10, probe holder, where sampled water is diverted upward for immediate contact with the probe sensor; 11, oxygen probe; 12, sampled water directed to other measuring equipment or to drain; 13, revolution-counter plate with gap to activate photocell (19); 14, drain from Level I to Level III; 15, drain from Level III to disposal; 16, ratchet-drive plate (with gap, as in the revolution-counter plate) to initiate recording of oxygen level just before full turn of the pickup arm; 17, 24-V ac electric motor, 1 rpm; 18, photocell to activate recording equipment just before pickup arm moves to the next channel; 19, photocell to stimulate electronic counter (once for every full turn of the pickup arm); 20, swivel joint (PVC tube over stainless steel tube with silicon lubricant; no leakage). The water sampler is about 41 × 41 × 38 cm, not including the probe holder and waste drain.

can be used for applications that require a longer response time, but only at the expense of less frequent recording for any one channel. A second photocell (Fig. 2, *19*) activates a counter circuit in the data compiler (Fig. 1, f), which stores a record of the number of full turns of the pickup arm. This information is recorded on tape in addition to the DOC readings.

The number of channels in the sampler described was determined by the number of fish tanks available for experimental work. When selecting the number of channels for such a sampler, two points should be considered: (1) The duration of a full cycle should not be a simple fraction of 1 h, or each source will be sampled at the same times each day. (2) Extra channels can easily be incorporated to provide for applications not included in the original experimental design.

This water sampling system has been in operation for a total of 6 months and has proved reliable. Early problems involved seizure of the hub that carries the pickup arm and ratchet wheel (Fig. 2, *7* and *8*); basing the hub on a sealed roller bearing resolved these problems. Under adverse conditions (dirty water, slow flow rate, or algal growth resulting from high water temperatures) the sample tubes need to be flushed frequently at high pressures.

With no fish in the tanks, the coefficient of variation of DOC between channels was about 0.2%, indicating an extremely low equipment bias between fish tanks, despite the range in length (1 to 12 m) of the sample tubes. Any error in the system (not to be confused with bias) is due almost exclusively to the oxygen-measuring

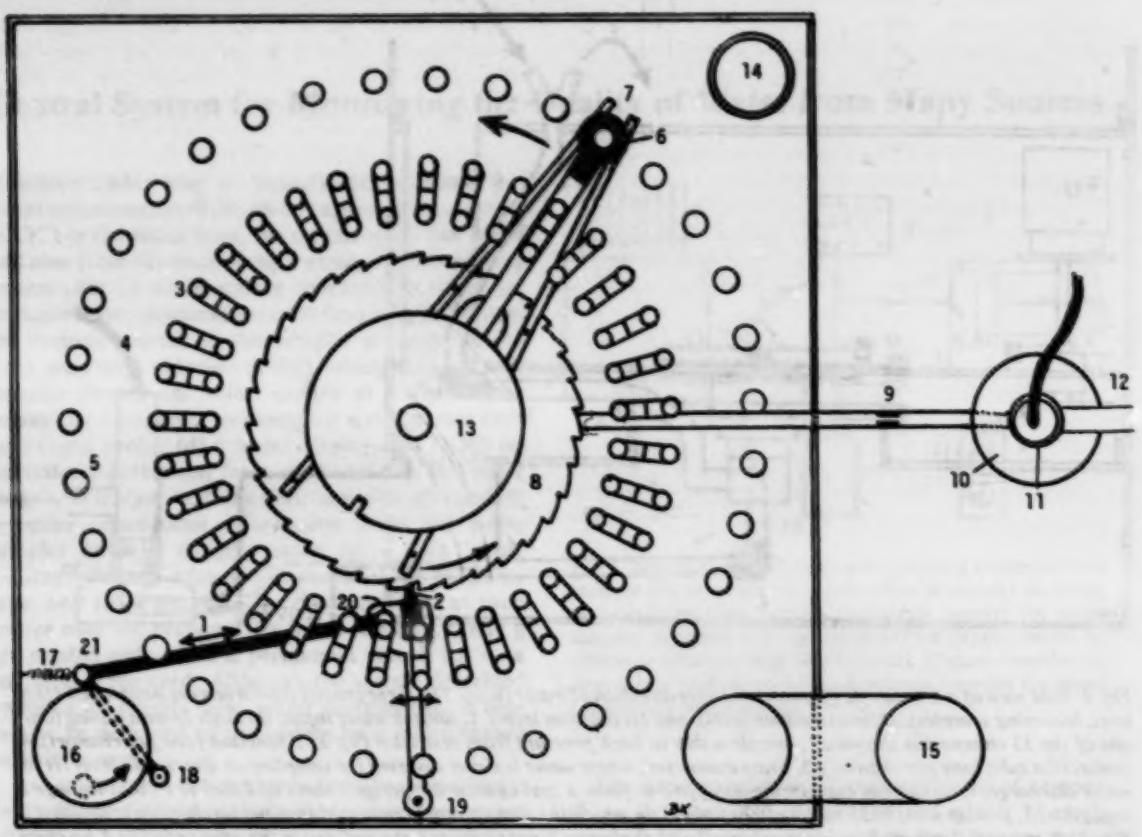

Fig. 3. Top view of water sampler (arrows indicate direction of mechanical motion). The motor and photocells are not shown in this view, items 3 to 16 are as given in the Fig. 2 caption, and only one of the 35 connection tubes (4) is shown. 1, ratchet mechanism, activated by an offset pin (16) once for every full turn of the motor, causing the pickup arm to move counterclockwise to the next channel; 2, sprung ratchet tooth; 17, ratchet return spring; 18 and 19, pivot points; 20 and 21, flexible joints.

equipment (Yellow Springs Instruments model 57 meter and matching probe YSI 5739). This equipment has been in continuous use for as long as 3 months. It has required only cleaning of the probe (by shaking the probe in the probe holder) and recalibration of the meter every few days; at recalibration the meter was usually about 0.5 ppm in error. For experimental applications, where comparisons between tanks are more important than absolute DOC values, this level of error would be acceptable if a single-probe system (like that described here) were used; however, when one probe per tank is used such an error level would generally be considered unacceptable and the probes should be recalibrated more frequently. Furthermore, if one probe per tank were used in a security (DOC-monitoring) application, labor costs would be high and the probability of a false alarm would be greatly increased.

The data compiler was designed and constructed at the Agricultural University of Norway (total cost, $700) as was the water sampler (total cost, $1,300).

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Accepted 22 June 1981

Rearing Lake Whitefish to Fingerling Size

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ABSTRACT: Lake whitefish (*Coregonus clupeaformis*) were reared on zooplankton after hatching until most fish readily accepted a dry diet by 13 weeks, when they averaged 40 mm (total length). Survival to 24 weeks was 4.0% and the fish then averaged 115.3 mm. The high mortality between 5 and 10 weeks was attributed to insufficient zooplankton caused by the high density of fry in the rearing containers; gill disease and fin rot also contributed to mortality.

Culture of lake whitefish (*Coregonus clupeaformis*) began at Northville, Michigan, in 1874 (Clark 1883). The fish were stocked as fry and few attempts were made to feed the fry and rear them to a larger size, although Stranahan (1898) mentions an early unsuccessful attempt. Extensive plantings of fry have been made in the Great Lakes, but no relation was found between the plantings and subsequent lake whitefish production (Van Oosten 1942; Lapworth 1956; Dymond 1957). Considerable research on rearing *Coregonus* spp. past the sac-fry stage has been done in Europe (e.g., Einsele 1963; Gunkel 1979; Fluchter 1980), but the limited comparable research on lake whitefish in North America has remained unpublished. Louella Cable (Great Lakes Fishery Laboratory, Ann Arbor, Michigan) raised lake whitefish to maturity at Northville, Michigan (1957 to 1963), as did James Reckahn (Ontario Ministry of Natural Resources) at South Bay Mouth, Ontario, in recent years.

In 1978 the Michigan Fish Producers Association (an organization of commercial fishermen) supported our research on the feasibility of rearing lake whitefish to fingerling size, as is commonly done with trout and salmon. The study was conducted in the Aquatic Laboratory of Lake Superior State College, located within the Edison Sault Hydroelectric Plant, Sault Ste. Marie, Michigan. The water supply for the laboratory is drawn from the power canal that originates in eastern Lake Superior.

Attempts to initiate feeding by the fry of *Coregonus* spp. on a dry diet have resulted in poor success or failure (Braum 1967; Gunkel 1979; L. Cable, unpublished data). We therefore decided to supply a live food source at first (zooplankton) and then to attempt to train the fish to take a commercial diet.

Egg Incubation

Eggs were taken from lake whitefish collected in Lake Superior near Brimley, Michigan, on several dates in November 1978. Eggs were fertilized and then incubated in standard 6-L hatching jars at temperatures ranging from 8°C at the start to 0.5°C. Hatching occurred in April and May 1979 and hatched fry averaged 14.3 mm total length.

Live Zooplankton Diet

Fry were reared in fiberglass baskets (window screen, 56 × 22 × 22 cm) placed in redwood troughs (305 × 25 × 20 cm) that contained about 100 L of water (Fig. 1). Each basket was filled to a depth of about 13 cm and held about 15 L of water.

Zooplankton, naturally present in the water supply, were concentrated about 140 times by filtering water through 363-μm plankton nets. Species composition of the concentrated zooplankton was essentially the same as that reported by Selgeby (1975) and consisted mostly of *Diaptomus* spp. and other copepods during the first weeks of fry rearing, with a greater variety of taxa, including cladocerans, appearing later.

Water with concentrated zooplankton flowed into each basket at a rate of about 0.5 L/min. Each basket was initially stocked with about 3,500 fry (233 fry/L), but this number was doubled to 7,000 (466 fry/L) 2 weeks later (26 May) as more fry hatched.

After nutrition and disease problems became apparent, the density of fry was reduced (during the 7th week) to 1,500 per basket (100 fry/L). During the 9th week of the experiment the fish were transferred into 300-L stock tanks and fed the Abernathy salmon diet six times daily in addition to zooplankton.

Fry mortality was less than 10% until 4 to 5 weeks (Table 1). Many fry began to feed on zooplankton within 24 h after hatching and virtually all were feeding several days after hatching. Fullness of the gut was judged by observing the amount of orange pigment (from zooplankton) present in the gut.

Mortality increased greatly after 5 weeks (Table 1) and continued at a high rate until about the 10th week.

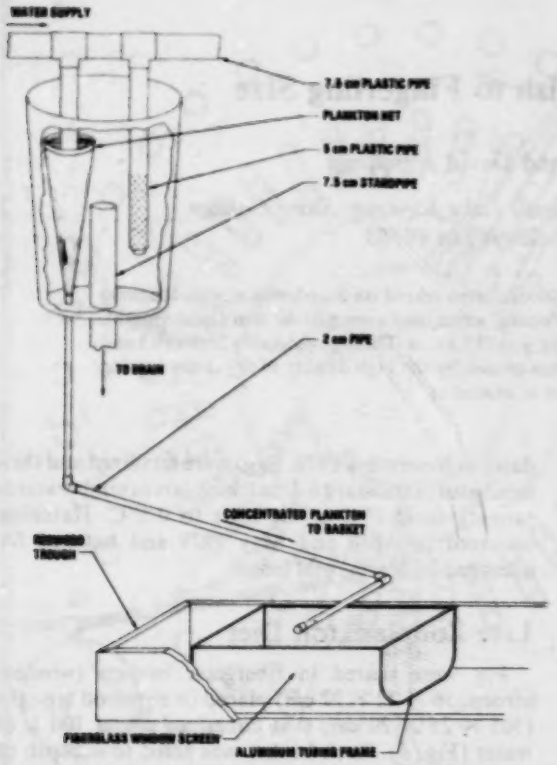

Fig. 1. Apparatus used to concentrate zooplankton and supply it directly to rearing baskets in redwood troughs.

During this period the guts of some of the fry contained gas bubbles that often left them helpless at the surface, and the guts also appeared to contain less orange pigment. Although the cause of the gas bubble problem went unrecognized at first, we now believe that the fry were swallowing gas bubbles in the water because they were not getting enough zooplankton. John and Hasler (1956) reported mortality of fry of cisco (*Coregonus artedii*) from swallowing gas bubbles, and Fluchter (1980) described the same condition for European whitefish when suitable food was lacking. Counts of zooplankton entering the baskets during this period indicated that an average of about 50 zooplankters per fish were entering the basket each 24 h. The question of whether this number is sufficient is discussed later in this report. When the fry were thinned to 1,500 per basket (100 fry/L) during the 7th week, the gas bubble problem disappeared and never reappeared.

Gill problems also contributed to the high mortality after the 5th week. The gills appeared swollen and mucus-covered, but microscopic examination revealed no pathogenic bacteria; we believe the problem was nutritional gill disease. (John Hgnath, fish pathologist at the Michigan Department of Natural Resources, examined some fish in August when the fish were 13 weeks old and confirmed the diagnosis.)

Mortality was low after the 10th week except for periods of high mortality during the 13th and 18th weeks. A dip treatment with salt relieved some of the symptoms of gill problems the 13th week, but the

Table 1. Survival and growth of lake whitefish reared at the Lake Superior State College Aquatic Research Laboratory, 1979.

	Live zooplankton diet		Frozen zooplankton diet		
Rearing period	Survival (%)	Average length (mm)	Survival (%)	Average length (mm)	Average water temperature (°C)
12 - 25 May	99.4	15.4	93.3	14.7	5.2
26 May - 8 June	97.3	18.4	91.4	15.6	6.8
9 - 22 June	91.2	19.9	36.0[a]	16.2	8.7
23 June - 6 July	34.3[b]	21.5			10.3
7 - 20 July	13.1[c]	27.7			14.3
21 July - 3 Aug.	11.6	38.8			18.3
4 - 17 Aug.	9.8[d]	44.0			17.7
18 - 31 Aug.	9.6	—			16.5
1 - 14 Sept.	8.8	63.5			16.9
15 - 28 Sept.	4.1	—			14.8
29 Sept. - 12 Oct.	4.0	—			13.8
13 - 26 Oct.	4.0	115.3			11.4

[a] Terminated because of poor growth and survival.
[b] Stocking density reduced.
[c] Transferred to 300-L tanks and fed the Abernathy diet (#1 granules).
[d] Transition from live zooplankton to dry food.

weakest fish died. During the 18th week fish were again given a salt dip and fed terramycin mixed with the dry diet because some showed symptoms of tail rot and external hemorrhaging (diagnosed by J. Hgnath). Survival to 24 weeks (26 October) was 4%, and among the fish still being kept at the laboratory less than 2% mortality has occurred in almost a year. Although the Abernathy diet was fed to the fish after 9 weeks, most fish did not eagerly accept the dry diet until they were 13 weeks old and averaged 40 mm (total length); a small proportion of the fish never accepted the dry diet. A Glencoe Mills dry diet was also tried. Hale (1970) used this diet for cisco (*Coregonus* spp.) fry and thought it deserved further testing, but we chose the Abernathy diet because the fish seemed to accept it better initially. After the fish began to accept dry food, they ate either diet readily.

Frozen Zooplankton Diet

About 9,000 fry received a diet of zooplankton (predominately *Diaptomus*) that had been frozen during the winter. About 3,000 fry (200/L) were stocked into each of three baskets and fed zooplankton frozen in ice cubes once or twice daily. Although the fry readily ate the zooplankton as it thawed and settled to the bottom, little or no bottom feeding was observed. A system to resuspend the dead plankton with intermittent jets of water from below the baskets was devised (such resuspension of food has been reported successful for striped bass [*Morone saxatilis*] by Lovell [1977]), and the fish fed readily on the resuspended material. However, the experiment was terminated after 6 weeks because of poor growth and survival.

Fluchter (1980) reported that although slowly frozen zooplankton and brine shrimp (*Artemia*) lacked a substance essential to the diet of European whitefish, the substance was retained by rapid freezing in liquid nitrogen. It seems probable that our fish suffered the same dietary deficiency as that reported by Fluchter, since our zooplankton was frozen slowly. Furthermore, the thawed plankton had a tendency to clump, providing a substrate for bacterial and fungal growth; when resuspended it caused fouling of the gills of some of the fry. Gill fouling of hybrid whitefish fry was observed by Garside and Christie (1962) when the fry were fed finely ground raw beef liver; they attributed the mortality to the inability of the fish to close their opercula.

Growth

About 40 fish were measured weekly to determine growth rates; fish fed live zooplankton grew 5.4 mm during the first 6 weeks (temperature range, 4.9 to 9.8°C), compared with an increase of only 1.9 mm for the fish fed frozen zooplankton (Table 1). The high mortality that occurred during the 5th to 10th weeks (when the fish fed live zooplankton probably had insufficient food) was not associated with any noticeable change in growth rate. After 24 weeks fish length averaged 115.3 mm; the water temperature reached a peak of 18.3°C during this period and averaged 12.9°C (Table 1).

The length-weight relation for 67 randomly chosen fish 15 to 136 mm long was

$$\text{Log } W = -5.49 + 3.19 \text{ Log } L.$$

The relation is approximate because the lengths and weights were mostly of fish that had been preserved in 10% formalin; however, the data points closely fit the regression line ($r = 0.994$) and live weights determined for 10 fish agreed well with the calculated weights.

Discussion

On the basis of laboratory studies, Braum (1967) concluded that food shortage was the most important factor causing mortality of coregonine larvae. Einsele (1963) reported nearly 100% survival of European whitefish under conditions of high zooplankton densities (at least 100/L) and sufficient illumination (more than 100 lux).

An adequate daily ration of zooplankton depends on water temperature and fish size. Braum (1967, 1978) calculated that *Coregonus wartmanni* 31 days old eat 265 copepods every 10 h at 15.6°C. Einsele (1941) reported a requirement of 150 zooplankton per day at 10°C for whitefish 14 days after first feeding. Hoagman (1973) calculated that laboratory-reared lake whitefish averaging 19.6 mm ate 18.6 copepods per day at 14.4°C.

An estimated 50 zooplankton per 24 h were potentially available to each of our fish during the 6th week, when high mortality occurred. Although our fish were older than those in some of the studies cited above, they averaged only 20 mm and the water temperature averaged only 9.8°C during that week. However, we believe the high density of fry in the holding baskets was the major cause contributing to high mortality. The oxygen in the water was always near saturation, ammonia was not a problem, and the troughs were well-lighted 24 h/day, but the density of the fish probably prevented a normal prey capture response. Much of the zooplankton passed through the baskets uneaten or died and settled to the bottom. L. E. Cable (unpublished data) worked with lake whitefish from Lake Erie and Lake Ontario and found survival rates of 9.3–18.7% to the end of the first year for fish from wild brood stock. The fish were fed various diets, beginning with brine shrimp nauplii, and were converted to a dry diet when they were 75 to 125 mm long.

Gunkel (1979) experimented with *Coregonus fera*, a European whitefish, and reported survival of 25–69% to 73 days. The fish were fed brine shrimp nauplii for 14 days, brine shrimp and dry food between 15 and 44 days, and only dry food after 45 days.

Hale (1970) reported about 90% survival of cisco to 6 months when the fish were held in 106-L tanks and fed brine shrimp; survival was less than 60% in 218-L tanks on the same diet. His fish were accepting dry food after 4 to 5 months.

Acknowledgments

The Michigan Fish Producers Association provided initial funding for this research. The senior author received undergraduate scholarship support from the National Fisheries Institute and additional funding from the Great Lakes Fishery Commission. Gale Gleason provided advice and assistance throughout the study. We thank John Hgnath for his diagnosis of disease problems, Peter Drake and James Reckahn for helpful advice, and Sally Linsley, Jeffrey Teska, and Janice Lowe for assistance in the laboratory and with data gathering.

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Accepted 8 September 1981

Feeding Success of Hatchery-reared Kokanee Salmon when Presented with Zooplankton Prey

Natural fish populations may be supplemented by stocking hatchery-reared fish; such stocking is often undertaken on the assumption that the stocked fish will successfully integrate into the existing fish community. To be successful, hatchery-reared fish must tolerate potential inter- and intra-specific competition not present in the hatchery, but because they have usually been fed commercially prepared food, they lack experience in capturing evasive natural prey. Studies of fish feeding have shown that success in capturing evasive prey is a function of experience (Confer and Blades 1975; Vinyard 1980) and thus hatchery-reared fish may initially be at a competitive disadvantage when stocked with wild fish populations. The purpose of the present study was to examine the efficiency with which hatchery-reared kokanee salmon (*Oncorhynchus nerka*) captured wild zooplankton.

Kokanee salmon brood stock are captured annually at Flathead Lake and their progeny are reared at the Sommers Hatchery by the Montana Department of Fish, Wildlife, and Parks. Most fish are released into Flathead Lake shortly after they swim up or they are used in stocking programs in other lakes; some are reared to fingerling size for various experimental programs. Experimental fish are maintained in raceways at the hatchery for several months and fed only Nelson's Sterling Silver Cup pelleted food before release.

We determined the capture success of naive and experienced hatchery-reared kokanee salmon by observing the fish feeding on zooplankton in a plexiglass aquarium 20 × 20 × 100 cm. The use of such long tanks provides a background determined primarily by the optical qualities of the water (Vinyard and O'Brien 1976; Confer and Blades 1975). Water temperature ranged from 9 to 16°C. Light intensities at the water surface ranged from 1000 to 1500 lux (15 – 26 microeinsteins/m^2 per second). Such levels of illumination are high enough so the fish can locate prey at their maximum reactive distances (Confer et al. 1978; Vinyard and O'Brien 1976).

Capture success was evaluated by introducing freshly caught zooplankton into an aquarium containing one or two kokanee salmon and counting their active strikes at the prey. After a known number of active attempts to capture prey were observed, the fish were removed from the aquarium and organisms in their stomachs were identified and counted. The zooplankton included *Daphnia thorata*, *Diaptomus* sp., *Cyclops* sp., and *Epischura nevadensis*. More than 2,150 attacks on copepods and 476 attacks on *Daphnia* by 33 kokanee salmon (mean standard length = 30.3 mm, SD = 6.2) were observed. The fish under observation were either unfamiliar with live zooplankton prey or had a known number of days experience consuming them in the laboratory. One day of experience consisted of feeding a group of fish freshly caught zooplankton in numbers such that each fish should have been able to consume a few hundred organisms. Fish were videotaped while feeding to enable analysis of the mechanics of the process.

Diets of wild kokanee salmon were determined from the stomach contents of fish trawled from Flathead Lake. The fish were collected in a midwater trawl with a 3.05-m square opening and a 1.27-cm (0.5-in.) mesh in the cod end, fished at a depth of 3 m. Zooplankton were collected as vertical haul samples from a depth of 10 m. All collections were made at about the same time (shortly after sunset), which A. D. Hanzel (personal observation) had previously determined to be the major period of feeding for kokanee salmon.

First attacks on living prey by naive kokanee salmon were characterized by relatively slow capture motions, similar to those they used to consume pelleted food. As fish gained experience they began to use a much faster and more vigorous attack technique. The body was much more strongly arched in preparation for rapid lunge at the prey. Janssen (1978) and Vinyard (in press) have observed similar differences in capture techniques exhibited by the alewife (*Alosa pseudoharengus*) and the Sacramento perch (*Archoplites interruptus*), respectively. Naive kokanee salmon were observed to capture *Daphnia* with 100% success, probably because of the weak evasion responses of most *Daphnia* (Drenner et al. 1978; Hall and Hyatt 1974).

Naive kokanee salmon had very low capture success for copepods (only 28% of the attacks observed on the first day of exposure; Table 1). The frequency of successful capture increased significantly with 1 day of

Table 1. Percent capture success (± SD in parentheses) in attacks on Diaptomus *by kokanee salmon with various levels of experience.*

Experience (days)	Capture success	No. of fish examined
1	27.9 (8.9)	11
2	36.5 (17.4)	6
3	39.9 (16.1)	8
4	39.6 (2.7)	8

experience ($P < 0.05$, test for equality of percentages, Sokal and Rohlf 1969) and later stabilized at about 40%.

This type of learned response to potentially evasive prey was observed by Confer and Blades (1975) with respect to the capture of copepods by sunfish (*Lepomis* sp.). The ability of sunfish to associate visual differences with evasion capabilities and to alter selectivities on the basis of learned differences in evasion has been demonstrated (Vinyard 1980). In a test of the retention of conditioned responses in juvenile hatchery-reared sockeye salmon (*Oncorhynchus nerka*), Tarrant (1964) determined that the fish could acquire conditioned responses rapidly (within 33 associations) and that such responses could persist for long periods (284 days).

Analysis of stomach contents of wild fish trawled from Flathead Lake indicated that these kokanee salmon are *Daphnia* specialists (Table 2); *Daphnia* averaged 98% of the prey in the stomachs of the sampled fish. Other observations of the diet of *O. nerka* have also shown a heavy reliance on *Daphnia* or other large cladocerans (Cordone et al. 1971; Hanzel 1973; Doble and Eggers 1978), or on dipteran larvae and pupae with seasonally dominant amounts of cladocerans (Efford and Tsumura 1973; Collins 1971; Chapman et al. 1967; Northcote and Lorz 1966). Goodlad et al. (1974) also noted that predation by kokanee salmon tended to fall most heavily on the larger of potential invertebrate prey. Values of electivity indices (Ivlev 1961) for kokanee salmon were positive for the large-bodied cladocerans *Daphnia* and *Leptodora* and negative for the small cladoceran *Bosmina* and for copepods (Table 2). Prey selectivity, as expressed in an electivity index, can result from evasion by the prey or selection by predators, or both. We observed that experienced kokanee salmon sometimes seemed to ignore copepods that were close enough for an attack, which suggests that both evasion and selection were at work. Electivity values indicated that kokanee salmon relied most heavily on the easily captured *Daphnia*.

When released into a lake inexperienced hatchery-reared kokanee salmon may initially be at a competitive disadvantage, which could affect growth rates. Hanzel (1975) has observed that recently stocked hatchery-reared kokanee salmon produced a check or false annulus in their otoliths. Bilton and Robins (1971) however, suggested that the production of such a check in the scales might also result from a period of rapid growth. Even after kokanee salmon become experienced planktivores, they are still relatively ineffective in capturing copepods. The observed dominance of cladocerans and relatively nonevasive dipteran larvae and pupae in the diet of *O. nerka* suggests that the growth of young kokanee salmon could be adversely affected when competitors are able to remove most of the nonevasive prey. Observations at Lake Tahoe (Morgan et al. 1978; Goldman et al. 1979) suggested that the growth of kokanee salmon was adversely affected by the disappearance of *Daphnia*.

Stocking success and growth rate of kokanee salmon are determined by three primary factors: cladoceran density, fish experience, and intensity of competition. Feeding experience should be most important for fish feeding on copepods. Theoretically, stocking success might be enhanced by feeding copepods to fish before release; however, feeding copepods to hatchery-reared fish on an extensive basis would prove difficult due to the generally long generation time for copepods (Hutchinson 1967) and the difficulty of culturing large numbers of planktonic copepods. Instead of attempting to educate naive fish, a more promising management tactic for stocking programs might be to schedule the release of kokanee salmon during periods when cladoceran abundance is greatest and competitor populations are lowest.

Acknowledgments

We thank Jack Stanford and the Flathead Research Group and John VanEngen of the Sommers Hatchery for their assistance in providing space and support for this work and the Montana Department of Fish, Wildlife, and Parks for providing fish and allowing

Table 2. Electivity values obtained from kokanee salmon (n = 7; average standard length = 113 mm; SD = 4.6) caught by midwater trawl, Flathead Lake, Montana, 24 July 1979. Average total numbers of prey found per fish: 2,174.

Item	Prey species					
	Daphnia	*Diaptomus*	*Epischura*	*Cyclops*	*Bosmina*	*Leptodora*
Electivity	+0.56	−0.97	−0.75	−1.0	−1.0	+0.03
Proportion of each prey type in fish stomachs	0.98	0.01	0.004	0.0003	0	0.01

our use of the *R. V.* Dolly Varden. The experiments were performed at the Flathead Lake Biological Station of the University of Montana.

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Accepted 8 September 1981

Seasonal Distribution of Esocids in a Power Plant Cooling Reservoir

The seasonal distribution of muskellunge (*Esox masquinongy*) and northern pike (*E. lucius*) in a power plant cooling reservoir was studied to facilitate their capture and use as brood fish. Brood stock are required for the production of muskellunge and northern pike as well as "tiger muskellunge" (muskellunge × northern pike hybrid). The species were introduced into the heated water of a power plant cooling reservoir to determine whether a brood stock could be developed there. We describe here the distribution of fish implanted with ultrasonic transmitters.

The cooling reservoir of the Big Stone Power Plant, a coal-fired 440-MW steam electric facility owned jointly by Montana-Dakota Utilities Company, Northwestern Public Service Company, and Otter Tail Power Company, is in northeastern South Dakota. The reservoir has an average surface area of 145 ha, maximum depth of 10 m, and mean depth of 4.9 m. Cooling water is withdrawn on the east side of a T-shaped dike and heated water is released on the west side; the rest of the reservoir serves as a mixing area (Fig. 1). There are 18 species of forage fish in the reservoir; muskellunge were stocked in the reservoir in 1978 and 1979.

Ultrasonic transmitters were surgically implanted into five muskellunge (45–47 cm total length) and five northern pike (52–95 cm) by methods described by Summerfelt (1972) and Crossman (1977), and terramycin was placed on the incision before the fish were placed in a recovery tank. Each fish was released into the reservoir after a recovery period of 30–40 min. Telemetry equipment (Donald L. Brumbaugh, Tucson, Arizona) included an ultrasonic receiver and directional hydrophone. The ultrasonic transmitters (16 × 60 mm; weight in water, 8 g) had a frequency of 75 kZh; pulse rates from 0.6 to 1.5/s distinguished individual fish.

Of the ten implanted fish, only one muskellunge and two northern pike survived and retained active transmitters for 1 year; they were tracked from July 1979 to July 1980. The distribution of the fish was determined by attempting to locate them 2 days each week during the winter and 3–5 days each week during other seasons. The muskellunge was located on 110 of the days, one northern pike on 121 days, and the other northern pike on 124 days. Fish locations were documented by triangulation with a sextant and were plotted on a map of the reservoir. Water temperatures were taken at 1-m depth intervals every other week at 10 stations in the reservoir with a temperature meter.

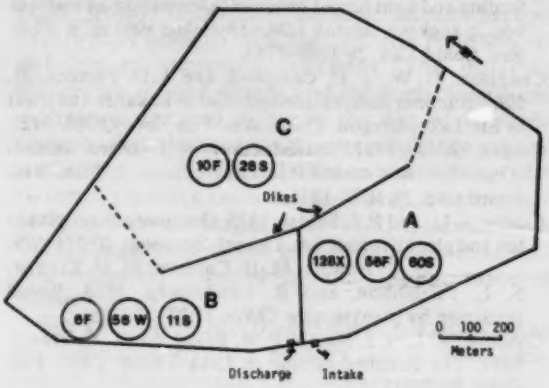

Fig. 1. Big Stone Power Plant cooling reservoir, South Dakota, showing intake area (A), discharge area (B), and mixing area (C), and locations of one muskellunge and two northern pike (data combined) on various days during summer 1979 (X), fall 1979 (F), winter 1979–80 (W), and spring 1980 (S).

The muskellunge and northern pike inhabited the intake area of the reservoir during the summer (9 July – 31 August) and the discharge area during the winter (2 December – 30 March; Fig. 1). In the fall (1 September – 17 November) and spring (4 April – 6 June), the fish spread throughout the reservoir. During summer, mean water temperature was 32.5°C (range, 27.5 – 39.5°C) in the discharge area and 29.5°C (range, 27.5 – 31.5°C) in the intake area (Table 1).

Table 1. Mean and (in parentheses) ranges for water temperatures (°C) in three areas of Big Stone Power Plant cooling reservoir, South Dakota, July 1979 – July 1980.

	Season[b]			
Area[a]	Summer	Fall	Winter	Spring
Intake	29.5 (27.5–31.5)	20.0 (18.5–21.5)	5.5 (2.0–11.0)	19.0 (15.0–23.5)
Discharge	32.5 (27.5–39.5)	22.0 (18.5–28.5)	11.0 (4.5–21.5)	24.0 (16.0–30.5)
Mixing	30.0 (26.0–34.5)	21.0 (18.0–23.0)	6.5 (3.0–12.0)	19.0 (12.0–26.0)

[a] See Fig. 1 for location of areas.
[b] Summer, 9 July – 31 August; fall, 1 September – 17 November; winter, 2 December – 30 March; spring, 4 April – 6 June.

During the winter mean temperature was 11.0°C (range, 4.5 – 21.5°C) in the discharge area and 5.5°C (range, 2.0 – 11.0°C) in the intake area. In fall and spring, temperatures ranged from 12.0 to 30.5°C in the reservoir.

Esocids were seasonally distributed in areas of the reservoir where temperatures were most favorable for their survival and growth. The upper lethal temperature for northern pike is 29.4°C and the optimum temperature for growth is 19 – 21°C (Casselman 1978); for muskellunge the respective temperatures are 32.2°C (Scott and Crossman 1973) and 20°C (Oehmcke et al. 1958; Buss 1960). The mean length of age I muskellunge stocked in the reservoir exceeded that reported for muskellunge in other waters in North America by 66–290 mm; age II fish exceeded reported lengths by 108–435 mm (Wahl and Applegate 1981).

Acknowledgments

We thank R. G. Johnson and N. N. Thomas, Otter Tail Power Company, for providing access and facilities at the cooling reservoir. This study was supported in part by Dingell-Johnson Project F-15-R and the South Dakota State University Agricultural Experiment Station.

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Accepted 18 June 1981

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Shielded-needle Technique for Surgically Implanting Radio-frequency Transmitters in Fish

Surgically implanted radio-frequency transmitters are preferable to external transmitters for experiments on fish in dense aquatic vegetation (Stasko and Pincock 1977). The performance of the implanted transmitter is improved by adding a protruding whip antenna, which results in (1) reducing the size of the transmitter; (2) providing a longer range for a given power output, and (3) requiring less critical tuning. Provision of an outlet for the antenna through the body wall has presented some problems, but Winter et al. (1978) described a technique in which a knitting needle was used to tunnel a cavity under the skin. However, we found this method somewhat difficult to use on fish with abdominal pelvic fins because of difficulty in tunneling past the pelvic girdle without piercing vital organs or the skin. To overcome this problem, we developed a shielded-needle technique to guide an antenna made of 24- or 26-gauge Teflon-coated conductor wire along the intestine under the pelvic girdle (Fig. 1).

A 3-cm incision was made along the linea alba anterior to the pelvic girdle on an anesthetized fish (Hart and Summerfelt 1975). A half-curved veterinary needle (Miltex No. 1291, size 0), shielded (to protect

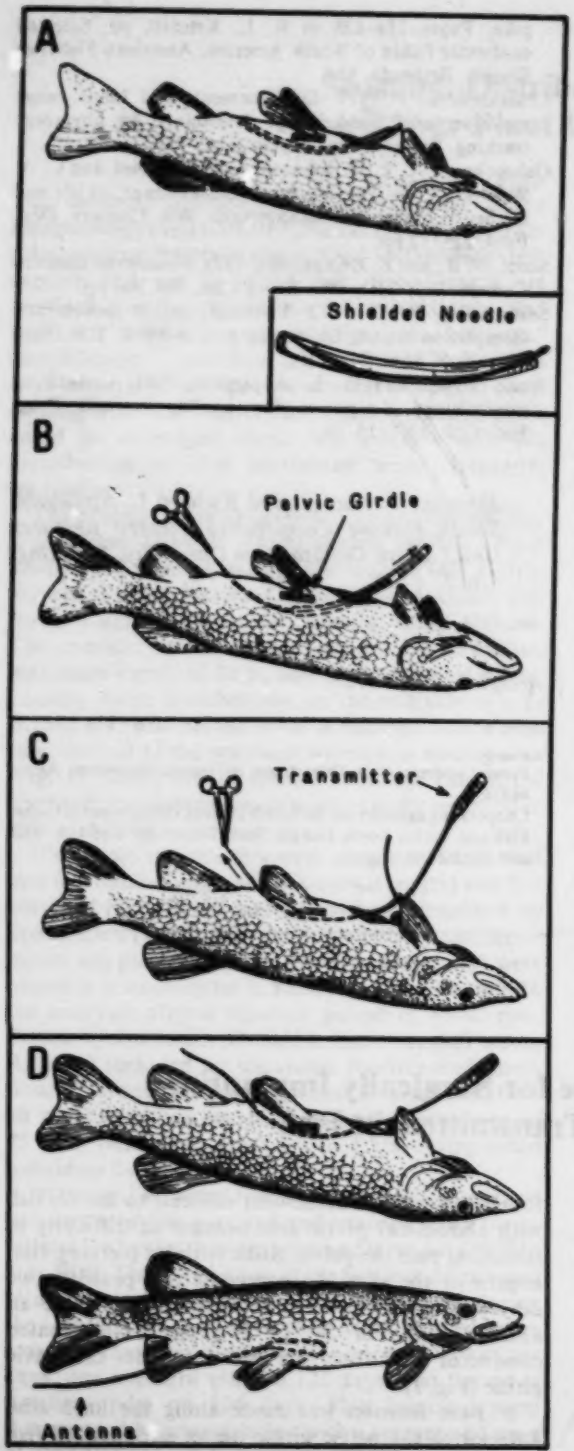

Fig. 1. A shielded-needle technique for surgically implanting radio transmitters with whip antennas in fish.

internal organs) with 0.5-cm plastic tubing from the tip to 1 cm in front of the eye of the needle (Fig. 1A), was inserted posteriorly along the intestine, past the pelvic girdle. Slight anterior tension was then put on the needle to free the tip from the inside wall of the plastic tubing and the needle was pushed forward and upward, piercing the skin midway (about 1 cm) between the pelvic girdle and the anus (Fig. 1B). The needle was held in place with needle-holder forceps, and the tubing removed by pulling it anteriorly over the eye of the needle. The whip antenna on a radio transmitter was threaded through the eye and the needle pulled posteriorly, out through the flesh (Fig. 1C). The transmitter was inserted through the original incision and positioned lengthwise in the peritoneal cavity (Fig. 1D). The incision and antenna exit hole were then sutured with noncapillary, nonabsorbable suture material.

This technique has been used on several species (Table 1), including some with thoracic ventral fins, because of the flexibility afforded for transmitter placement. The data show that fish lived a minimum of 28 days, and one was observed more than 18 months after the transmitter was implanted, indicating that major damage to the internal organs had not occurred. Best results were obtained when fish were captured, tagged, and returned immediately to an open lake environment. Fish returned to a confined area did not survive as well as those in the open waters. Using a similar technique, Kuechle et al. (1981) also reported mortalities in both control and test groups of white suckers (*Catostomus commersoni*) in a confined area.

Problems commonly encountered with surgically implanting tags in small fish noted by Prince and Maughan (1978) included poor recovery, damage to organs, and slow healing. All of our fish recovered within 20–25 min from the MS-222 anesthetic and were swimming normally. In our studies, tissue damage was decreased when we used smaller transmitter-to-fish size ratios. Although conditions may vary among species, we believe the transmitter sizes used for the small muskellunge (*Esox masquinongy*) and northern pike (*E. lucius*) used in the present study (Table 1) are close to the upper limit of transmitter-to-fish size and weight ratios; the transmitter weight was about 1.7% of the fish weight. Three of four flathead catfish (*Pylodictis olivaris*) held in water at 4°C died before the incision healed. Similar problems were not, however, reported by Hart and Summerfelt (1975), who worked with this species. Walleyes (*Stizostedion vitreum vitreum*) and surviving white suckers were healed within 30 and 45 days, respectively (Kuechle et al. 1981).

Our technique enables the transmitter to be positioned anywhere in the peritoneal cavity, which reduces healing problems and the damage to vital organs that may occur when the transmitter is placed

Table 1. Summary of data on use of the shielded-needle technique for planting radio-frequency transmitters in fish.

Species	Fish			Transmitter					Observation period (days)[a]	Mortalities (no.)	Environment[b]
	Range			Weight (g)							
	Lengths (cm)	Weights (kg)	No. tagged	Dry	In H_2O	Length (cm)	Diameter (cm)	Antenna length (cm)			
Muskellunge (*Esox masquinongy*)	85–122	4.8–12.7	14	65	40	6.9	3.2	61	547	1	L
Flathead catfish (*Pylodictis olivaris*)	67–86	3.9–8.2	4	65[c]	40	6.9	3.2	46	65	3	HP
Walleye (*Stizostedion vitreum*)	63–73	3.2–4.5	3	21[c]	12	5.0	1.8	46	23	0	HP
Muskellunge	50–53	0.8–1.0	9	21	12	5.0	1.8	31	75	1	L
Northern pike (*Esox lucius*)	50–59	0.7–1.1	4	21	12	5.0	1.8	31	75	0	L
Walleye	41–49	0.5–0.9	11	9	4	3.5	1.1	23	28	4	ESC

[a] Observations of the larger muskellunge (85 – 122 cm) continued for 18 months; studies of the smaller muskellunge (50 – 53 cm) and northern pike are continuing.
[b] L = lake; HP = holding pond; ESC = experimental stream channels.
[c] Dummy transmitters.

at the incision or anterior to it. In contrast, the technique of Winter et al. (1978) does not permit transmitter placement posterior to the incision without a 180° bend in the antenna, since the tunnel begins at the posterior end of the incision. The shielded-needle technique also appears to be less traumatic than the Winter technique and requires substantially less force on the needle than tunneling between tissue layers, especially near the pelvic girdle.

Acknowledgments

We thank the Monticello Ecological Research Station and the Minnesota Department of Natural Resources for their cooperation in these studies. We especially acknowledge the assistance of Robert Strand, Larry Gates, and Bruce Gilbertson. This work was primarily supported by grants from the U.S. Environmental Protection Agency (No. R805290-01-0) and the U.S. Department of Energy (No. DE-AC02-76EV01332) to the University of Minnesota.

Accepted 23 June 1981

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Effects of a Diet of Bullfrog Larvae on the Growth of Largemouth Bass

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ABSTRACT: The effects of a diet of bullfrog (*Rana catesbeiana*) larvae versus a diet of minnows and suckers on the growth of largemouth bass (*Micropterus salmoides*) were compared. The dry weight intake of bass fed the tadpole diet for 25 days was similar ($\bar{x}$ = 1.41 g/day) to that of bass fed minnows and suckers ($\bar{x}$ = 1.57 g/day), but those fed tadpoles had a significantly smaller change in condition factor ($\bar{x}$ = 0.04 and 0.11, respectively). The diets were then reversed (bass previously fed tadpoles were fed minnows and suckers, and vice versa) for 14 days. During this period the daily intake of the group fed minnows and suckers was significantly larger ($\bar{x}$ = 1.81 g/day) than that of the group fed tadpoles ($\bar{x}$ = 0.81 g/day). The condition factor of bass on the fish diet improved, whereas bass on the tadpole diet lost weight. It is suggested that bullfrog larvae are suboptimum forage items for largemouth bass.

Bullfrogs (*Rana catesbeiana*) have a relatively long larval stage (2-3 years in the northern part of their distribution) and thus overwinter as tadpoles in permanent bodies of water where predacious fishes, such as the largemouth bass (*Micropterus salmoides*), coexist. However, field accounts of bass consuming bullfrog larvae are rare (Dubets 1954; Lewis et al. 1974). Lewis et al. (1961), using artificial enclosures, found that bass consumption of bullfrog tadpoles was sparing in comparison with their consumption of golden shiners (*Notemigonus crysoleucas*). Lewis and Helms (1964) suggested that bullfrog larvae are highly vulnerable to largemouth bass predation in artificial enclosures. Their study reported that although bullfrog tadpoles were the forage items taken most frequently, the bass on this diet gained no weight, whereas the bass that fed on fingerling bullheads (*Ictalurus* spp.) consumed a smaller amount of forage but gained weight. Kruse and Francis (1977) offered a partial explanation for such a finding when they demonstrated that bullfrog tadpoles were relatively unpalatable to largemouth bass: they found that largemouth bass preyed only sparingly on bullfrog tadpoles when offered a variety of anuran larvae, but fed on bullfrog larvae when not given an option. Kruse and Francis (1977) concluded that this unpalatable nature of bullfrog larvae serves as a predator-deterrent mechanism which allows for coexistence with aquatic predators in permanent bodies of water. These observations suggested that further study on the use of bullfrog larvae as forage for largemouth bass was needed.

Materials and Methods

Our experiments were conducted from August to October 1977. Largemouth bass were obtained by electrofishing from several reservoirs in Lancaster and Seward Counties, Nebraska, transported to the laboratory, and maintained in 80-L aquaria. Each aquarium was filled with aged tap water that was aerated and filtered continuously with under-gravel and charcoal filters. The aquaria were shielded so that bass could not observe fish in adjacent tanks. The experimental aquarium room was maintained at a temperature of 21 ± 2° C and a photoperiod of 14L:10D (0800–2200 h). Bass were fed minnows and crayfish until the experiments began.

Bullfrog larvae (stages 27 through 38, Gosner 1960) were obtained by seining from several reservoirs in Lancaster County. The tadpoles were maintained in a 240-L aluminum tank and fed boiled lettuce. Prey fishes were minnows (*Notropis* spp., *Semotilus* spp., *Pimephales* spp.) and suckers (*Catostomus* spp.) that were seined from small creeks within the Salt Valley drainage (Lancaster County), maintained in a 240-L aquarium, and fed commercial fish food pellets.

After a 48-h fast, 14 bass were weighed to the nearest 0.1 g and measured to the nearest 1.0 mm (total length). The bass were allowed to reacclimate for 24 h before the treatment diets were administered. Seven randomly chosen bass were fed tadpoles (Group 1, diet A), and seven were fed minnows and suckers (Group 2,

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diet B). The daily tadpole rations were lightly blotted with an absorbent paper and weighed to the nearest 0.1 g. The prey fish were weighed to the nearest 0.1 g in a pre-tared container of water. Both diets were offered ad libitum between 1700 and 1900 h. After 24 h, prey items that were dead, regurgitated, or untouched were removed, weighed, and subtracted from the oroginal daily allotment to determine daily intake.

The first experimental period lasted 25 days, at which time the bass were fasted for 48 h, and lengths and weights were again recorded. The bass were then allowed to reacclimate (without food) for 5 days. A second experiment was then initiated to determine if a reversal in the diets would produce an opposite trend in the growth of the two test groups. To test for this effect the diets were switched: bass that had initially been fed diet A (tadpoles) were fed diet B (prey fish) and vice versa. After 2 weeks on this dietary regime and a 48-h fast, lengths and weights were again recorded and the bass were released.

Kirk (1967) conducted chemical and caloric analyses of bullfrog tadpoles and fry of green sunfish (*Lepomis cyanellus*) and demonstrated that the wet weight of tadpoles consists of 90.5% water, whereas water comprises only 79.3% of the wet weight of sunfish. Average daily intake dry weight was calculated on the basis of these figures. Although green sunfish were not the forage fish in the present experiments, Love (1957) reported that fish flesh averaged 80% water. Kirk (1967) also showed that, on the basis of wet weight, tadpoles have only half the caloric content (0.29 kcal/g) of green sunfish fry (0.58 kcal/g). We used these data to estimate caloric intake.

Lengths and weights were used to calculate the condition factor (cf. Le Cren 1951) of each largemouth bass before and after each experiment. Variables of interest for both experimental groups over both time periods included: (1) the average daily intake (in grams) of wet and dry weights; (2) average caloric intakes; and (3) the average change in condition factor. Mean values were compared by using the Student's *t* distribution.

Results and Discussion

Mean initial condition factors of the bass fed the two diets (Table 1) were not detectably different ($P > 0.05$). Comparison of the change in mean condition factors of the fish on each of the two diets in both of the time periods showed that a significantly greater positive change was produced by diet B (prey fish) than by diet A (tadpoles; $P < 0.05$ in experiment 1 and $P < 0.001$ in experiment 2). Comparison of mean daily wet weight intake for the two diets indicated a significantly larger intake by bass in group 1 on diet A than by those in group 2 on diet B during the 25-day experiment ($P < 0.05$). However, when the wet weight intake was converted to dry weight and caloric value, similar weights and calories were consumed by fish in both groups during experiment 1 (Table 1). Since diets were offered ad libitum, bass fed

Table 1. Growth comparisons of two groups of seven bass fed diets of bullfrog tadpoles (diet A) or minnows and suckers (diet B) during two consecutive experiments (1 = 25 days; 2 = 14 days). One standard deviation is given below the mean. Calculated t-values from comparisons of means are given where appropriate.

Experiment, group, and diet	Weight (g)		Total length (cm)		Condition factor		Average daily intake (g)		Average caloric intake of forage consumed (kcal)
	Initial	Change	Initial	Change	Initial	Change	Wet weight	Dry weight	
Experiment 1									
Group 1	276.0	24.0	27.2	0.4	1.34	0.04	14.81	1.41	4.34
Diet A	98.3	21.6	3.5	0.24	0.06	0.05	6.42	0.61	1.88
Group 2	267.5	53.7	26.7	1.0	1.36	0.11	7.60	1.57	4.43
Diet B	90.3	23.2	2.7	0.24	0.11	0.08	2.23	0.46	1.30
					t=0.57	t=1.84*	t=2.81**	t=0.57	t=0.10
Experiment 2									
Group 1	300.0	30.7	27.6	0.3	1.38	0.07	8.72	1.81	5.08
Diet B	117.7	28.3	3.5	0.05	0.05	0.06	4.57	0.95	2.66
Group 2	321.2	-4.6	27.7	0.2	1.47	-0.06	8.56	0.81	2.51
Diet A	106.9	3.9	2.6	0.24	0.06	0.03	2.74	0.26	0.80
						t=5.53***	t=0.08	t=2.68*	t=2.45*

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

diet A appeared to compensate for the lower caloric value of the tadpoles by doubling the wet weight intake. Similar observations were reported by Kirk (1967), who suggested that the food intake of bass is governed by caloric value rather than by bulk, as has been demonstrated for goldfish (*Carassius auratus;* Rozin and Mayer 1961).

When the diets were reversed, the wet weight intake of both groups was similar; however, on the basis of dry weight or caloric content, group 1 fed diet B (prey fish) consumed significantly more than group 2 fed diet A (tadpoles). Results from experiment 2 are somewhat harder to interpret, since the initial condition of group 2 bass (previously fed diet B) was probably better than that of group 1 fish that had been fed diet A (tadpoles) during experiment 1. All bass on diet A in experiment 2 lost weight, even though they all continued to consume tadpoles.

The relatively poor nutritional value of bullfrog tadpoles to largemouth bass appears clear from results of experiment 1. The change in condition factor of largemouth bass fed tadpoles was significantly less than that of bass fed prey fish. Results from experiment 2 showed that when the diets were reversed, the trend in weight change for individual fish was also reversed, substantiating the conclusion that the differences in growth were related to the diets of the bass.

On five occasions bass that were maintained on diet A were observed to ingest a tadpole (killing it three of the times) and then reject it. Bass also regurgitated portions (usually partly digested) of their intake of tadpoles on eight occasions. Kruse and Francis (1977) observed similar behavior. Bass maintained on diet B (prey fish) were never observed regurgitating. The fecal material of the bass fed diet A was also observed to be markedly different than that of the bass fed diet B. The bass fed tadpoles produced voluminous amounts of diffuse fecal meterial, whereas those fed diet B produced compact pellet-shaped fecal material which sank to the bottom of the aquarium.

The exact cause of the lesser weight gain achieved by bass fed bullfrog tadpoles may be related to any combination of the following: (1) reduced food intake because of the relatively unpalatable nature of tadpoles (which occurred in the second experiment but not in the first), (2) reduced assimilation of food intake due to high fecal or excretory loss, and (3) reduced nutritional quality of a diet of tadpoles.

The data presented here suggest that bullfrog tadpoles are probably not a quantitatively important dietary item of largemouth bass. The absence of bullfrog tadpoles in stomachs of bass taken in the wild (Dubets 1954; Lewis et al. 1974) adds credence to this supposition. It is unlikely, however, that bass abstain from foraging on bullfrog tadpoles in small farm ponds or hatchery ponds where more palatable prey may be lacking, since largemouth bass are known to consume bullfrog larvae in such areas (Bremer 1965). It is suggested that bullfrog tadpoles are suboptimum forage items for largemouth bass.

Acknowledgments

We thank the University of Nebraska–School of Life Science Robert M. Wotton fund for financial assistance during a portion of this study, Paul Neimeier for help in collecting tadpoles, Donn Rodekohr of the Nebraska Game and Parks Commission for help in collecting bass, and John D. Lynch for providing laboratory facilities and criticizing the manuscript.

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Accepted 18 June 1981.

Comparative Hatching Success of Lake Trout Eggs in Lake Michigan Water and Well Water[1]

Although lake trout (*Salvelinus namaycush*) have been stocked in Lake Michigan since 1965 and spawning activity has been widespread each fall since 1971 (Wells and McLain 1973), extensive sampling has produced fry on only a few occasions (Wagner 1980; Jude et al. 1981) and has failed to locate any yearling or older lake trout (Great Lakes Fishery Laboratory 1978; Kutkuhn, in press). This apparent reproductive failure of the lake trout caused speculation that contaminants (e.g., DDE, PCB's) in the water and biota of Lake Michigan might be a contributing factor. Previous studies have indicated that water source might influence the survival to hatch of lake trout eggs. Both Stauffer (1979) and Mac et al. (1981) incubated eggs in water from two different sources (laboratory and lake) and reported reduced hatching success for eggs incubated in lake water. The present study was designed to examine the influence of water from southern Lake Michigan on the survival of lake trout eggs by comparing the hatching success of eggs from the same source incubated in water from Lake Michigan or from the laboratory well. (An additional objective of the present study had been to raise the fry past the yolk-absorption stage, to observe whether lake water caused increased mortality similar to that reported by Berlin et al. [1981], who observed reduced survival of fry exposed to simulated Lake Michigan levels of PCB's and DDE for 6 months. However, a power failure at the field site resulted in the death of all fry before yolk absorption.)

The field site was located inside the Municipal Water Filtration Plant, Muskegon, Michigan. Untreated water from Lake Michigan flowed from the Plant's intake into a 189-L fiberglass reservoir and (through polyvinyl chloride pipe) to four, 37-L glass tanks.

During incubation, eggs rested on Nitex screen inside containers made from acrylic tubes (Berlin et al. 1977). One egg container was suspended with a monofilament nylon line in each of the four tanks. Each tank was wrapped with black plastic and covered with a Styrofoam lid to limit exposure to light. At the laboratory site (Great Lakes Fishery Laboratory), experimental equipment was identical with that used at Muskegon.

Incubation temperature at both sites followed the ambient temperature cycle of Lake Michigan (12.2–1.1°C) except for the first 13 days of incubation. During this period equipment problems caused us to maintain the average water temperature in the laboratory at 7.3°C (range, 7–8°C) while the average temperature at the field site was 10°C (range, 12.2–9.1°C).

Adult lake trout in spawning condition were collected in Lake Michigan off Saugatuck, Michigan, on 8 November 1978. Pooled eggs and milt, from 11 females (total length, 691–834 mm) and 12 males (total length, 566–844 mm), were held on ice and the eggs were fertilized on shore at the field station (Saugatuck) about 30 min after collection. The fertilized eggs were allowed to water harden in charcoal-filtered tap water for about 1 h before they were transported by car to the two study sites. At each site, we transferred 150 eggs into each of four acrylic egg containers, using a glass tube (8 mm ID) equipped with a rubber squeeze bulb.

Total water hardness at the field site averaged 140 mg/L as $CaCO_3$ (range, 132–150 mg/L); alkalinity averaged 114 mg/L (range, 108–128 mg/L); and pH ranged from 7.7 to 8.2. The total hardness of well water at the laboratory averaged 442 mg/L, average alkalinity was 354 mg/L, and pH was 7.7.

Mean percent hatch for eggs in Lake Michigan water at the field site was 35 (range, 31–38) — significantly lower (χ^2, $P < 0.001$) than the mean of 73.7 (range, 71.3–75.3) at the laboratory. Examination of the eggs and sac fry indicated that no bacterial or viral pathogens were involved in the lowered hatching success of the eggs in Lake Michigan water.

Although the higher water temperature at the field site for the first 13 days of incubation may have affected the egg mortality there, we still observed a difference in survival between the two sites from day 14 on. Eggs at the field site suffered 43% mortality, whereas eggs in the laboratory had only 25% mortality after day 14.

Our findings agree with previously published research. Stauffer (1979), who incubated eggs from both lake and hatchery origin in egg enclosures submerged in Lake Michigan waters of Grand Traverse Bay, reported only 37 and 34% survival to the swim-up stage for lake and hatchery eggs, respectively, compared with about 60% for eggs from the same sources taken in the same year, and incubated in a hatchery. Mac et al. (1981) showed that the survival to hatch of eggs of lake trout collected from Lake Michigan near Saugatuck incubated at lake temperatures in laboratory well water was 61%, in contrast to 46% for eggs incubated in water from Lake Huron. Contaminant

[1] Contribution 584 of the Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, Mich.

analysis of fish collected from Lake Michigan and Lake Huron in 1972 showed the presence of DDT and PCB's in samples from both lakes, but the concentrations were lower in fish from Lake Huron (Michigan Department of Agriculture 1974). This finding suggests a possible correlation between the level of contaminants in each of the lakes and the survival of lake trout eggs incubated in water from the lakes.

We conclude that the observed differences in hatching are probably attributable to chemical constituents of water from Lake Michigan (e.g., chlorinated hydrocarbons, metals, other industrial and agricultural chemicals). The importance of these chemicals in the overall reproductive failure of lake trout is not known.

Acknowledgments

We thank personnel of the Muskegon Filtration Plant for the use of the facility and for the water chemistry data they provided; and Toshio Yasutake, National Fisheries Research center, Seattle, Washington, for conducting pathological examinations of the lake trout fry.

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Accepted 29 July 1981

Water Flow Controller

A requirement for accurate measurement and control of water flow into 1-m² fry tanks led to the development of a cistern-based flow controller (Fig. 1) that has proved to be more accurate in use than commercially available flow meters.

The controller is constructed of polyvinyl chloride components throughout. A piece of clear tubing (0.5 m long, 63 mm OD; 3 mm wall thickness) is blanked off at one end to form a vertical cistern that is mounted or hung at the water supply pipe (Fig. 2). Water enters the cistern from the supply line through a clear flexible hose. The end is plugged and drilled to retain large foreign matter in the water supply. Water enters the tank through a perforated screw-on cap at the bottom of the cistern. To ensure an even flow into the fish tank, 5 cm of small-bore tubing is glued into the cap.

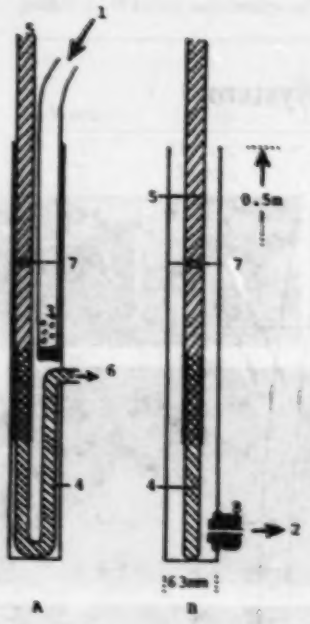

Fig. 1. Rear (A) and side (B) views of the controlled delivery cistern: 1, input from water supply; 2, output to tank; 3, filter; 4, U tube, 20-mm OD; 5, adjustment tube, 25-mm OD, which fits over the U tube; 6, overflow to waste or reuse; 7, overflow port; 8, perforated screw-on cap.

The water level stabilizes at an overflow port cut in a pipe 25 mm in diameter. This pipe fits over a similar 20-mm pipe that leads to an overflow outlet. By sliding the larger pipe up or down over the smaller pipe, the water level can be set at any point over a range of about 20 cm. Large changes in flow rate can be accomplished by changing the screw-on cap, and fine adjustment by altering the water level in the cistern. For four different caps the correlation between flow rate and water level averaged 99.87%, with no significant deviation from linearity.

These four caps were used to obtain standard delivery rates to a test cistern; 40 additional caps were then calibrated. For each cap a simple regression equation of the form flow rate = a + (b × water level) was calculated to determine water level for any desired flow rate. In practice, these equations can be used directly or tables of height versus flow rate can be used. A cap with a 7-mm outlet hole gives a flow rate of between 5 and 7 L/min, depending on water level chosen.

Fig. 2. Controlled delivery cistern in use. The cistern is hung from the last of a series of 90° bends that are attached to the water supply line. The pipe at the bottom left corner carries overflow water that does not enter the tank.

We have used 33 of these cisterns continuously for more than 1 year at the Research Station for Salmonids, Sunndalsøra. Material costs were about $10 per unit.

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Accepted 22 June 1981

Holding Atlantic Menhaden in a Closed System for Environmental Research

A study with Atlantic menhaden (*Brevoortia tyrannus*) was conducted to evaluate the potential effectiveness of angled screens, behavioral barriers, and fish pumps in preventing fish losses at power plant intakes. Fish had to be held for about 3 months under known conditions to meet the study objectives. A search of available literature and communication with other researchers indicated that there was no clear definition of holding facilities or conditions necessary to maintain Atlantic menhaden in a closed system, using synthetic sea salt. In this note I describe the holding facility used and the conditions that were monitored, and indicate the relative success of the effort.

Two holding pools were required to contain the numbers of fish needed for testing. Each pool operated as an independent closed-cycle system and was equipped with a biological filter, physical adsorption filter, and a rapid sand pressure filter (Fig. 1). Two pools with a capacity of 9,462 L (2,500 gal), 366 cm (12 ft) in diameter and 91 cm (3 ft) deep, were assembled on a concrete floor (Fig. 2E). The blue vinyl pool liners were throughly scrubbed (without using a cleaner), rinsed, and dried. W. F. Hettler (National Marine Fisheries Service, Beaufort Laboratory, personal communication) suggested that the provision of a dark-colored sidewall might keep menhaden from abrading their jaws on the perimeter of the pool.

Fig. 2. Holding system components: A, rapid sand pressure filter; B, biological filter; C, center drain (hollow plexiglass chamber); D, biofilter components (plastic grid, nylon screen, gravel); and E, holding pool with physical adsorption filter (lower left).

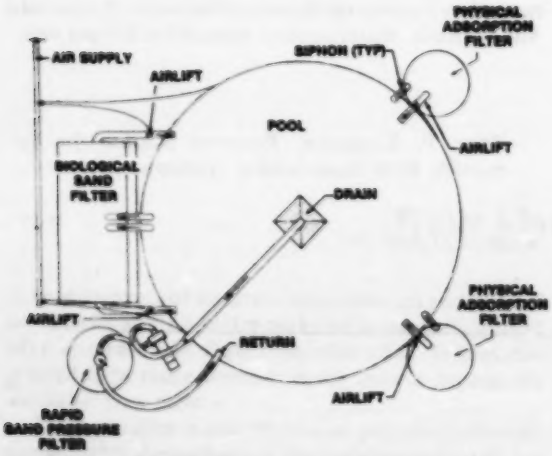

Fig. 1. Schematic diagram of holding system.

Accordingly, a strip of black rip-stop plastic was attached with polyvinyl chloride (PVC) pipe cement to the base of the blue pool liner sidewalls, extended up over the rim of the pools, and secured with steel bands to keep the liners taut. The pools were then filled with dechlorinated tap water previously adjusted in an 18,925-L (5,000-gal) conditioning sump to a salinity of 6.1 °/₀₀ (Table 1) with Instant Ocean synthetic sea salt.

The biological filters (Fig. 2B) were constructed from 1.27-cm marine plywood. The filter plates were plastic grid covered with nylon screen, which supported the filter medium (a 10-cm layer of graded gravel; grains, 2–3 mm; Fig. 2D). Water was circulated through the filters by a siphon and airlift consisting of 5-cm (2-in.) PVC pipe. As air was introduced into the bottom of the lift tubes through an airstone, filtered water was displaced upward and into the holding pool and reoxygenated in the process as unfiltered water was drawn onto the filter surface through the siphon.

Table 1. Weekly summary of mean physical and biological variables.

Week	Date (1978)	Physical variables				Fish growth[a]	
		Salinity (°/₀₀)	Temperature (°C)	pH	Ammonia nitrogen (mg/L)	Fork length (mm)	Weight (g)
1	27 Aug.	6.1	19.1	7.6	0.02	—[b]	—[b]
2	3 Sept.	5.9	19.8	7.6	0.02	54 (39–66)	2.4 (0.9–4.1)
3	10 Sept.	5.4	16.8	7.6	0.40	58 (45–67)	2.7 (1.2–3.8)
4	17 Sept.	6.8	16.7	7.6	0.02	—[b]	—[b]
5	24 Sept.	6.7	15.8	7.6	0.02	55 (43–66)	2.5 (1.2–3.7)
6	1 Oct.	6.7	16.1	7.3	0.02	57 (46–68)	2.7 (1.3–4.9)
7	8 Oct.	4.4	13.0	7.2	0.02	59 (49–70)	2.9 (1.8–4.5)
8	15 Oct.	5.5	12.2	7.2	0.02	—[b]	—[b]
9	22 Oct.	6.0	13.0	7.2	0.02	—[b]	—[b]
10	29 Oct.	5.9	11.4	7.2	0.02	—[b]	—[b]
11	5 Nov.	5.8	11.5	7.2	0.02	62 (52–80)	3.6 (2.1–7.2)

[a] Fork lengths and weights are given as means and (in parentheses) ranges. They are based on subsamples of 50 fish removed from population on the days tests were conducted.
[b] Not experimented with in test flumes, therefore, not weighed and measured.

The turnover rate through the filters was 4 L/min per square meter of filter surface area (9 L/min, total).

Because of space limitation, the biological filters did not have the same surface area as the pools (pool = 10.4 m² vs. filter bed = 2.2 m²), as recommended by Spotte (1979). Although a larger biofilter would have increased the carrying capacity of the system, the size used maintained an acceptably low range of ammonia nitrogen (0.02 to 0.4 mg/L; Table 1). Bacteria seed from a conditioned filter bed was obtained to expedite the conditioning process, since time was not available to culture a new filter bed.

The physical adsorption filters, which served to remove dissolved organics and maintain water clarity, consisted of two 114-L (30-gal) plastic pails per pool, each filled with 23 kg of bone charcoal (Fig. 2E). An airlift and siphon in each filter circulated water at a rate of 8 L/min (16 L/min, total).

Fiberglass rapid sand pressure filters (sand grains 0.05 – 0.1 mm) connected to 1.5-hp Marlow plastic pool pumps were used to remove solid fish wastes and to circulate pool water at a rate of 242 L/min (Fig. 2A). The walls of a hollow plexiglass chamber were drilled with about 100 10-mm holes and covered with nylon screen (Fig. 2C); this chamber was attached to the suction pipe to distribute the inflow at the filter intake and create a uniform low-velocity flow field. The filtered effluent was discharged at the pool surface through an adjustable pipe fitting that created a circular flow in the pool. The menhaden were observed to orient to this discharge and the resulting flow pattern.

Air was supplied by a pair of Gast oilless air compressors, one on line and one in reserve. An oxygen bottle was connected to a normally closed electric solenoid that, if needed in the event of a power failure, would fail open and supply the airlifts until normal power was restored.

Ambient conditions determined water temperatures and pH; only salinity was controlled. About 10% (1,135 L) of the water in the holding pool was discharged daily during backflushing of the rapid sand pressure filter, and makeup water was pumped in from the conditioning sump.

Young-of-the-year Atlantic menhaden were collected in Buzzards Bay near Falmouth, Massachusetts, by Sea Plantations, Inc., Salem, Massachusetts. These fish were held at Salem in (dilute) seawater at 28 °/₀₀ salinity for about 1 week before transport. Before each shipment, the salinity of the holding water was gradually lowered by dilution with fresh water. Two lots of 2,000 fish were shipped to the laboratory during the study, and their condition before shipment was good. The fish were transported (for about 3 h) in two rectangular tanks (0.7 m³) lined with vinyl inserts and aerated with oxygen.

The first lot of menhaden was transported on 28 August 1978. On arrival, the holding pool water (5 °/₀₀ salinity, 17.5°C) was pumped into the transport tank water (12 °/₀₀, 17°C). The fish were thus acclimated to the holding pool water for 30 min before being transferred to the holding pool. The transport tank water for the second shipment of fish on 26 September 1978 was at 5 °/₀₀ salinity and 13°C; the fish were again acclimated for 30 min, this time to 5 °/₀₀ salinity and 16°C. To reduce the initial handling stress during unloading at the laboratory, the menhaden were transferred in pails from the transport tank to the holding pool.

Mortality from transport and handling was low. The two shipments of 2,000 fish had 47 (2.3%) and 28 (1.4%) dead, respectively, after 24 h. About 300 fish

per shipment had bruised mouth parts from transport, but this condition cleared up during the first week of holding.

The menhaden were fed pulverized Tetramin TB 104 dry flake food. Food was sprinkled on the water surface 6 to 8 times a day. As the food began to sink, the school would pass through the concentrate and consume it before it could reach the pool bottom. Once a day the fish were fed frozen adult brine shrimp. They were sustained with Tetramin and *Artemia*, exclusively, for the 3-month study.

Fish lengths and weights were measured on the days tests were conducted, and water quality was monitored daily. A weekly summary of the biological and physical variables is given in Table 1.

During the study 4,000 Atlantic menhaden were held at the laboratory, 2,000 for 90 days and 2,000 for 60 days. The range of lengths at the beginning of the study was 39 to 66 mm; by the end of the study it had increased to 52 to 80 mm (Table 1). Fish were observed to feed and no disease was apparent. The fish that died exhibited signs of scaling or bruises, indicating that holding-tank mortality was associated with handling. Overall mortality at the end of the study, calculated from the daily mortality record in the main holding tanks, was about 20%.

Various rectangular shaped test flumes, separate from the circular holding pools, were used to test the response of the menhaden to certain flow velocities and diversion devices. Mortality was observed to increase among the menhaden subjected to a no-flow condition for an extended period in the rectangular test flumes, whereas the menhaden removed from the flumes immediately after flows ceased experienced less mortality. When flows were terminated the fish would disperse, swim into sidewalls and corners, and injure themselves. This type of behavior did not occur among the menhaden in the holding pools, where the circular flow appeared to enhance survival. Further observations showed that menhaden being moved between test models and holding tanks could be transferred without tempering from 3 to 8 °/₀₀ salinity and back again without any apparent stress reaction.

Although the research study with the menhaden lasted only 3 months, about 300 fish were then held over the winter for a total of 8 months at temperatures as low as 1.9° C. Mortality for the overwintering group was about 30%. These fish were mixed in with juvenile striped bass (*Morone saxatilis*) that were being tested in other studies, and were fed only frozen adult *Artemia*.

The results of the present study indicate that menhaden that are collected and introduced into the laboratory can be maintained in a closed system and handled in moderation for research purposes. Researchers desiring to use a system similar to that desribed can probably increase the carrying capacity by using a biological filter larger than the one described here.

Acknowledgments

The studies were funded by the Empire State Electric Energy Research Corporation and conducted at the Alden Research Laboratory, Holden, Massachusetts, by Stone and Webster Engineering Corporation. I thank Robert L. Keene for his invaluable assistance, and Thomas A. Biffar and Edward P. Taft III for their constructive reviews of the manuscript.

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Accepted 18 June 1981

Effect of Delivery Method and Antigen Preparation on the Production of Antibodies Against *Aeromonas hydrophila* in Channel Catfish

Immunization by hyperosmotic infiltration (HI) is an important tool in controlling and preventing enteric redmouth disease (ERM) in trout (*Salmo* and *Salvelinus* spp.) and vibriosis in salmon (*Oncorhynchus* spp.). Croy and Amend (1977) reported as much as a 100% reduction in mortality in sockeye salmon (*O. nerka*) immunized against *Vibrio anguillarum* with HI in comparison with non-immunized controls. Wildlife Vaccines, Inc. (11457 48th Avenue, Wheat Ridge, Colorado) reported an 84% mortality reduction (7.91% to 1.25%) in fish immunized by HI against ERM versus non-immunized controls in a field experiment involving 23 million fish. Several other reports substantiate the efficacy of bacterins delivered by HI in coldwater fish (Gould et al. 1979; Egidius and Anderson 1979; Amend et al. 1980; Moody 1978; Lannan 1978). However, no product has been developed for the immunization of channel catfish (*Ictalurus punctatus*) or other warmwater fish. The present study had two major objectives: (1) to determine if hyperosmotic infiltration or an aerosol spray of a hyperosmotic bacterin is a practical alternative to injection for the delivery of an *Aeromonas hydrophila* bacterin in channel catfish and (2) to compare the efficacy of various bacterin preparations when delivered by injection, immersion, and aerosol spray.

A virulent isolate of *A. hydrophila* obtained from gizzard shad (*Dorosoma cepedianum*) was cultured on brain heart infusion agar for 18 h at 37°C. Cells were harvested and washed three times in 0.85% NaCl and then heat-inactivated at 60°C for 1 h. Before heat-inactivation, a sample was aseptically removed for enumeration of cells by the standard plate count technique. The stock suspension was subsequently split into three fractions: one was left as a whole-cell preparation, a second was alternately frozen at -90°C and thawed three times, and the third was sonicated at 70 W for five 20-s intervals with an Artek Sonic Dismembranator (using the intermediate tip). All bacterin preparations were preserved by the addition of formalin to a final concentration of 0.1%. Sterility of all bacterin preparations was confirmed before use by inoculation into thioglycollate broth (Difco Laboratories) incubated at 37°C for 48 h.

Ten groups of 10 Auburn strain channel catfish (average weight, 238 g per fish) were placed in 4.58-m^3 circular plastic pools receiving pond water at a rate of 2 L/min. Fish were fed Auburn #3 sinking pellets at a rate of 3% of body weight per day. After a 2-week acclimation period, three groups of fish were immunized by each of the following delivery procedures: (1) intramuscular injection of 0.1 mL of bacterin (10^8 cells/mL) suspended in an equal volume of Freund's complete adjuvant, (2) hyperosmotic infiltration by means of a 3-min immersion in a 3% NaCl solution containing 2×10^{11} cells/mL, and (3) an aerosol spray of a solution identical to the one used in the immersion procedure. Of the fish in each procedure, one group received the whole-cell preparation, another the freeze-thaw preparation, and the third the sonicated preparation. A final group of 10 fish served as non-immunized controls. Immunized groups of fish received a booster application 10 days later. Late afternoon water temperatures ranged from 27°C at the outset of the study to 25.5°C at the conclusion.

A month after being boosted, fish were bled from the caudal peduncle and serum agglutinin levels were determined in microtiter plates, using a washed, heat-inactivated, whole-cell preparation as the antigen. After a log transformation of the titer values (Lefkovitz and Pernis 1979) data were analyzed by Duncan's method for comparing treatment means (Sokol and Rohlf 1973).

Injection of bacterin with adjuvant resulted in significantly better antibody production, regardless of the preparation method. Sonication produced the most effective bacterin; freeze-thaw and whole-cell preparations elicited much poorer antibody responses in all three delivery methods (Figs. 1, 2). The fish that

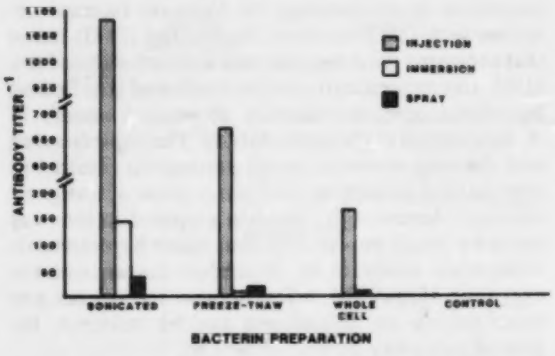

Fig. 1. Average of reciprocal of antibody titers of channel catfish to which different preparations of Aeromonas hydrophila *bacterin were administered by subcutaneous injection, immersion (HI), and aerosol spray. (Fish immersed in the whole-cell preparation were lost just before the end of the study.)*

received the whole-cell preparation by immersion died due to interruption of the water flow 2 days before the study was completed.

Although delivery of the sonicated bacterin by HI or by spray of the HI solution resulted in significantly ($P > 0.95$) higher titers than either the whole-cell or freeze-thaw preparations delivered by immersion or spray methods, injection of any of the three preparations was significantly more effective than delivery by the other methods. Agglutinin titers of fish to which whole-cell and freeze-thaw preparations were administered by immersion and spray were not significantly different from those of control fish (Fig. 2).

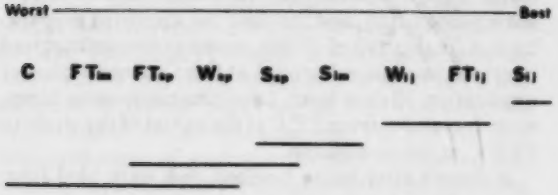

Fig. 2. Results of Duncan's multiple range test for comparing each treatment mean to all others. Treatments underscored by the same line are not significantly different (P = 0.95), and ranked from poorest to best at eliciting agglutinating antibodies as indicated. C = control, S = sonicated, Ft = freeze-thaw, W = whole cell, ij = injected, im = immersion, sp = spray.

The finding that the hyperosmotic procedure elicited the greatest antibody response when a sonicated bacterin was used indicates that release of antigenic material from the cell wall (probably endotoxin) is important in maximizing the humoral immune response with this procedure. Sutherland (1973) stated that sonicated gram negative cell walls are reduced to a highly fragmented state, and we confirmed that finding by microscopic examination of stained smears of *A. hydrophila* in the present study. The triple freezing and thawing of the bacterial suspension resulted in only partial disruption and less release of antigenic material. Accordingly, bacterin prepared in this way elicited a much poorer response. Since hyperosmotic infiltration seems to be dependent on an osmotic exchange of material, it follows that the smaller and more soluble an immunogen can be rendered, the greater its uptake by the fish will be.

Our results indicate the superior effectiveness of injection over immersion for inducing a humoral response in channel catfish. Although antibodies are of great importance in an immune response, there is not necessarily a direct correlation between antibody titer and protection from pathogen challenge. Other factors of the cellular immune system also play a role, and in combination with even low antibody titers, can effectively protect against virulent challenge. With this in mind, and considering the relative ease of delivering a bacterin in hyperosmotic solutions, we conclude that HI seems to be a practical alternative to injection as a method for vaccinating channel catfish. Further refinements in delivery methods regarding time of exposure, salt concentration, antigen concentration and preparation, as well as studies to determine protection against challenge, may further increase the effectiveness of HI as a method for immunizing channel catfish.

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Accepted 8 September 1981

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Further Observations on a Fish Production System that Incorporates Hydroponically Grown Plants

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ABSTRACT: Water temperature was elevated and maintained in two of three fish production systems which incorporated biofiltration and hydroponics. Channel catfish (*Ictalurus punctatus*) were stocked in indoor fish tanks and fed to satiation once daily. Tomatoes (*Lycopersicon esculentum*) were planted in outdoor hydroponic tanks. The study was conducted during the 1979 growing season. Afternoon water temperature averaged 25°C in the control system and 28°C in both experimental systems. The temperature in the control unit varied with the ambient diel cycle, whereas the temperature in the two experimental units remained constant. The mechanics of the system were satisfactory and the plants showed no adverse effects associated with the heated water. The total fruit yield (in kilograms per plant) was similar in all systems: control, 8.9; experimental A, 9.1; and experimental B, 9.1. Growth of the yearling fish was enhanced by the constant elevated temperature. The respective average rates of growth from 15 May to 22 June in the control system and experimental systems A and B were 0.96, 1.47, and 1.06 g/day per fish.

Lewis et al. (1978) reported on a fish production system in which water quality was maintained by biofiltration and plants were grown hydroponically. Additional evaluation of this system, and a new design of the system, are given in Lewis et al. (in press). In the present study we continued the evaluation of the new design and examined the effects of constant elevated temperature on channel catfish (*Ictalurus punctatus*) and tomatoes (*Lycopersicon esculentum*). The latter consideration is of particular importance, since channel catfish exhibit maximum growth at 28 to 30°C (Andrews et al. 1972). However, there is some question concerning the effects of constant elevated temperature on the performance of tomatoes, which are superior plants for use in such a system. Went (1944) reported that both better growth and fruit set were produced in tomato plants by warm days and cool nights than by constant temperature. Also, Went (1957) and Resh (1978) suggested that the optimal temperature for growth of tomatoes might vary with the stage of plant development.

Methods and Materials

The system used in the present study included a 190-L fish culture tank equipped with an agitator for aeration (Fig. 1). The indoor fish tank was coupled to a revolving-plate biofilter modeled after the design of Lewis and Buynak (1976). The biofilter consisted of three compartments (total surface area, 2.7 m²) and was connected to a sedimentation-flotation tank which, in turn, was linked to a pair of outdoor hydroponic tanks that were filled with gravel (5 – 10 mm size) and positioned at different levels. Each hydroponic tank measured 3.05 m long, 0.51 m wide, and 0.25 m deep. The lower tank was connected to a water-equalizing reservoir which compensated for water lost through evaporation and transpiration. The total volume of the system was about 750 L.

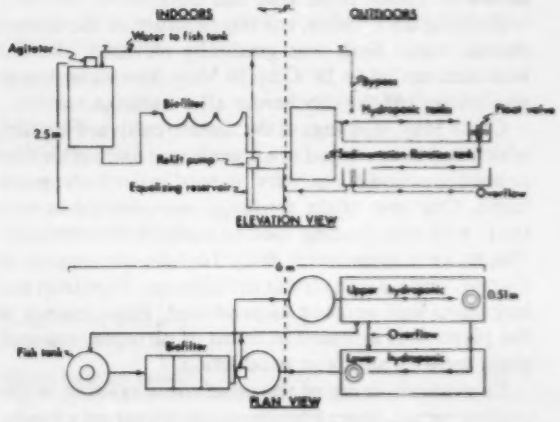

Fig. 1. Design for fish-production system incorporating biofiltration and hydroponically grown plants.

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Water was continuously pumped to the fish tank from the equalizing reservoir at 11 L/min, resulting in a volume change in the fish tank every 17 min. Water flowed by gravity from the fish tank through the revolving-plate biofilter into the sedimentation-flotation tank, and from that tank into the upper hydroponic tank. The upper tank filled to a level just below the surface of the gravel in about 5 min; a float valve then tripped and the water drained from the upper tank into the lower tank and the connected equalizing reservoir. The upper hydroponic tank emptied in about 3 min and then the drain valve reseated. As the water was pumped to the fish tank, the water level in the lower hydroponic tank and equalizing reservoir dropped until the upper hydroponic tank again filled to the level needed to trip the float valve.

One control system and two replicate experimental systems were used. Both fish tanks of the two experimental systems were placed in one 570-L water bath with a thermostatically controlled submersible electric water heater. The temperature of the water in the control unit varied with the ambient diel cycle, but the temperature of the water in the two experimental units remained constant within the limits of the thermostat (± 1 Celsius degree). Heat loss was minimized by insulating the outside of the hydroponic tanks that were supplied with heated water with a 2.54-cm layer of Styrofoam.

Yearling channel catfish (average, 46 g) were stocked into each fish tank at a density of 5.6 kg/system on 30 April 1979. The fish were fed to satiation once daily and the amount fed (dry weight) was recorded. The feed used was Purina Trout Chow in the form of a floating pellet. Fish were individually weighed several times during the study to determine growth rates. They were observed daily for mortality and signs of disease. When mortality was high, fish of similar sizes were added to replace those that had died.

Starting on 17 May, the temperature in the experimental water bath was gradually elevated; the fish were acclimated to 28°C by 26 May. The water heater was turned off the day before all weighings.

On 18 May, seedlings of the tomato cultivar Floradel, which had been started in a greenhouse and acclimated to outdoor conditions, were planted in the hydroponic tanks. One row of six seedlings was planted in each tank, with wire fencing used to support the tomatoes. Plants were monitored daily for the occurrence of disease, pests, and nutrient deficiencies. Ripe fruit was harvested and weighed as produced. Performance of the plants was assessed in terms of nitrogen removal, plant growth, and fruit production.

To evaluate some of the conditions existing in the system, we monitored temperature, dissolved oxygen, alkalinity, and pH. Water temperature was recorded each afternoon with a YSI Model 46TUC telethermometer. Dissolved oxygen was measured twice each week with a YSI model 54 oxygen meter. Alkalinity was monitored weekly by titration and pH was measured twice each week with a Corning model 12 pH meter. To insure the required nutrients for the plants, potassium, calcium, and magnesium levels were monitored every 2 weeks with a Beckman model 1301 atomic absorption unit. The daily addition of makeup water (aerated well water) was recorded.

The following variables were monitored with particular reference to evaluation of the effects of elevated temperature on the performance of channel catfish and production of tomatoes: total ammonia nitrogen (NH_4-N + NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), health and growth rate of fish, and growth and fruit production of tomatoes. Total ammonia nitrogen (NH_4-N + NH_3-N) was measured twice each week with an Orion 901A ionalyzer. Nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) were recorded weekly, using a Hach kit.

The study was terminated on 28 September, and all fish, plants, and fruit mass were weighed. Several plants were air dried to determine average dry weight.

Results

The standing crops of fish for the control system and experimental systems A and B at the time of harvest were 49.5, 64.7, and 27.9 kg of fish per cubic meter of volume of the fish tank, respectively; or 0.04, 0.06, and 0.02 kg of fish per square meter of biofilter surface (for both revolving plate and hydroponic beds); or 0.78, 1.03, and 0.44 kg of fish per tomato plant.

The seasonal afternoon temperature in the control fish tank averaged 24°C from 15 May to 22 June, 26°C from 22 June to 22 July, and 25°C from 22 July to 28 September; the average water temperature in the experimental fish tanks averaged 28°C from 15 May to 28 September (Table 1). On 28 May, the water pump and aerator in experimental system B malfunctioned, resulting in an oxygen depletion and the loss of 61 fish. With the exception of this oxygen depletion, the lowest oxygen concentration observed during the study in any fish tank effluent was 4.2 mg/L (53% saturation) in experimental system A. Dissolved oxygen in the fish tank effluent of the control system was never below 5.0 mg/L (60% saturation). During most of the experiment the oxygen concentration in all systems remained above 70% saturation (Table 2).

Average alkalinity was similar in all systems: 241, 249, and 246 mg/L (as calcium carbonate) for the control system and experimental systems A and B, respectively. Fluctuations in pH were similar in all systems, ranging from 7.7 to 8.4. The average daily makeup water for the control system and for experimental systems A and B was 6.0, 5.8, and 6.0% of

Table 1. Effects of elevated temperature on channel catfish growth in three systems combining fish production and hydroponics.

Variable	Time interval (1979)											
	15 May – 21 June (38 days)			22 June – 21 July (30 days)			22 July – 28 Sept (68 days)			Total study (136 days)		
	Control	A[a]	B[b]	Control	A	B	Control	A	B	Control	A	B
Fish tank temperature[c] (°C)	24	28	28	26	28	29	25	27	27	25	28	28
Daily growth rate (g/day per fish)	0.96	1.47	1.06	1.35	0.01	0.85	1.97	2.47	1.62[d]	1.57	1.65	0.86
Food conversion (food fed/ weight gain)	1.22	1.18	1.38[e]	1.52	—	—	1.61	1.40	2.25[d]	1.43	1.29[f]	1.82[f]
Mortality (%)	2	2	2(54)[g]	0	33	9	3	0	18[d]	3	11	12

[a] Experimental system A.
[b] Experimental system B.
[c] Average daily afternoon temperature.
[d] 18 August – 28 September.
[e] Adjusted for added fish.
[f] Time interval 22 June – 22 July excluded.
[g] Includes oxygen depletion mortality.

each total volume, respectively; that is, total water loss from transpiration and evaporation was about 6% per day.

In all systems, magnesium and calcium concentrations were consistently above the requirements for tomatoes based on the "minimum balanced nutrient regime" calculated by Lewis et al. (1978) from Asher and Ozanne (1967). Deficiencies of potassium and boron were suspected on 29 July, when blackening of several plant shoots was observed. Potassium levels at that time were about 1.0 mg/L in all systems. Nutrient supplements of all essential elements were added weekly after 1 August to the end of the study and the evidence of the deficiency disappeared.

Levels of total ammonia nitrogen (NH_4-N + NH_3-N) in the influent to the fish tanks of the control system and experimental systems A and B averaged 0.18, 0.21, and 0.15 mg/L and levels in the effluent averaged 0.70, 0.81, and 0.55 mg/L, respectively.

The highest measured concentration of nitrite nitrogen (NO_2-N) in the influent to the fish tanks was 0.65 mg/L in the control system (Table 2), which is well below the 96-h mean tolerance limit of 7.5 mg/L (reported as 24.6 mg/L nitrite) set by Konikoff (1975).

The growth rate of the seedling shoots (centimeters per day per plant) was slightly better in the experimental systems than in the control system during the first 13 days after planting: control, 0.55; experimental A, 0.66; and experimental B, 0.66. However, based on the results of an *F*-test, these growth rates were not significantly different ($P > 0.05$). Also, from 21 May to 18 June, the total percentage of nitrate nitrogen

Table 2. Mean effects of constant elevated temperature on water quality and plant production in three systems combining fish production and hydroponics (1979).

Variable	Systems[a]		
	Control	Experimental	
		A	B
Fish tank temperature[b] (°C)	25 (15–31)	28 (22–31)	28 (23–32)
Fish tank effluent dissolved oxygen (% saturation)	83 (60–100)	78 (53–91)	83 (62[c]–93)
Fish tank influent total ammonia nitrogen (NH_4-N + NH_3-N; mg/L)	0.18 (0.00–0.86)	0.21 (0.00–1.54)	0.15 (0.00–1.02)
Fish tank influent nitrite nitrogen (NO_2-N; mg/L)	0.05 (0.00–0.65)	0.03 (0.01–0.10)	0.04 (0.00–0.22)
Upper hydroponic influent nitrate nitrogen (NO^3-n; mg/L)	11.8 (1.6–43.5)	20.7 (4.0–84.0)	11.9 (0.9–52.5)
Total plant biomass[c] (roots + shoots; g/plant)	603.1	641.9	608.2
Total root biomass[d] (g/plant)	107.9	114.0	173.8
Total shoot biomass[d] (g/plant)	495.2	527.9	434.4
Shoot-to-root ratios per plant	5.6	5.3	3.0

[a] Range values given in parentheses.
[b] Average daily afternoon temperature.
[c] Excludes oxygen depletion.
[d] Dry weights.

(NO_3-N) removed per pass through the two hydroponic tanks of each unit was slightly better in the experimental systems than in the control system: control, 3; experimental A, 6; experimental B, 5. Flowering of all plants began on 4 June. The first ripe fruit was harvested the second week of July in all systems. No plant disease was evident, and insignifigant damage was noted from hornworms and the western locust.

The total yield of plant biomass (dry weight of roots and shoots) was greatest in the experimental systems (Table 2). The average dry weight of root biomass at harvest was also highest in the experimental systems. Experimental system B was not protected from the afternoon sun, and the plants in the upper hydroponic tank of this system were scalded during August, resulting in lower total shoot mass and in significant differences (*F*-test and Duncan's multiple range test; $P < 0.05$) in the shoot-to-root ratios in this system compared with those in the other two systems (Table 2). The total fruit yield per plant (kilograms of ripe + green fruit) was slightly better in the experimental systems; control, 8.9; experimental A, 9.1; and experimental B, 9.1.

The average weight gain for fish in the control system from 22 July to 28 September at a mean water temperature of 25°C in the fish tank was 1.97 g/day per fish (Table 1). This compares with an average gain in experimental system A of 2.47 g/day per fish at a mean temperature of 27°C, and in experimental system B from 18 August to 28 September of 1.62 g/day per fish at a mean temperature of 25°C.

After the 22 June weighing, the fish in both experimental systems developed a bacterial infection (*Aeromonas* sp.) when the water temperature was elevated rapidly and held at 28°C. No diseased fish were observed in the control system. The first mortality due to the pathogen in the experimental systems occurred on 25 June. The growth rates from 22 June to 22 July were drastically reduced by the disease (Table 1). Fish in experimental system B experienced a higher incidence of disease than those in experimental system A after 22 July. On 18 August, after failing to eliminate the pathogen in both experimental systems by 1-h treatments of Furacin (10 mg/L, 100% active) and sodium chloride (0.4%), all fish were removed from experimental system B and its fish tank chlorinated (200 mg/L). This system was flushed, treated with Furacin, and restocked with a new batch of fish. The water temeprature in both experimental systems was then gradually elevated to 25°C (rather than the 28°C previously mentioned). Immediately after stocking, the new fish in experimental system B showed signs of aeromonal infection and were treated with Furacin and sodium chloride as described above. This same treatment was administered to these fish 24 h later. The fish in both experimental systems slowly recovered and signs of the disease were not evident by 27 August.

The average weight gain for fish in the control system from 22 July to 28 September at a mean water temperature of 25°C in the fish tank was 1.97 g/day per fish (Table 1). This compares with an average gain in experimental system A of 2.47 g/day per fish at a mean temperature of 27°C, and in experimental system B from 18 August to 28 September of 1.62 g/day per fish at mean temperature of 25°C.

Disregarding the mortality from the oxygen depletion, total mortalities were 3, 11, and 12% in the control system and experimental systems A and B, respectively (Table 1).

Discussion

The present study posed three questions, namely: whether the mechanical features of a new design of the closed system involving biofiltration and hydroponics functioned satisfactorily; whether constant elevated water temperature would be detrimental to the performance of the tomato plants in the hydroponic tanks; and whether constant elevated temperature would adversely affect channel catfish.

The system design constitutes an advance in simplicity and reduction of initial investment over that proposed by Lewis et al. (1978). The principal simplification is that one continuously operating relift pump is used in the new design, as opposed to two pumps operating intermittently in the previous design. The new system retains the basic features of the original: preventing exposure of the water to sunlight and providing for reciprocating movement of the water in the hydroponic beds. The earlier design (Lewis et al. 1978) incorporated an elevated reservoir with a gravity drain to the fish tank, which protected the fish during power outages. Since the elevated reservoir was eliminated in the new design, an emergency electrical power source in the form of a gasoline generator with automatic power transfer should be incorporated into a permanent installation of the new design.

The constant elevated water temperature did not adversely affect the performance of the tomatoes. In fact, the uptake of nitrate nitrogen and fruit production was slightly better in the experimental systems than in the control system, which operated at a lower and fluctuating temperature.

As was to be expected, the fish exhibited better feeding and increased growth at the higher temperatures. Nevertheless, both fish populations held at elevated temperatures developed a serious infection (*Aeromonas* sp.) after the 22 June weighing and handling. Not until the temperature had been lowered from 28 to 25°C was it possible to control the infection. On the basis of the findings of Beem (1978),

we theorize that the elevated temperature was detrimental to the immune response of the fish and favorable to *Aeromonas* sp.

In summary, the new design is superior to that proposed by Lewis et al. (1978). The elevation of at least root temperature did not adversely affect the performance of the tomatoes. The constant elevated temperature of about 28°C resulted in increased growth of fish, but appeared to make them more susceptible to bacterial infection when handled.

Acknowledgments

The authors thank John Yopp, Botany Department, and John Stahl, Zoology Department, Southern Illinois University–Carbondale, for their helpful suggestions while the research was being done. This report is based on R. J. Sutton's research for an M.A. degree in the Department of Zoology and the Graduate School, Southern Illinois University–Carbondale.

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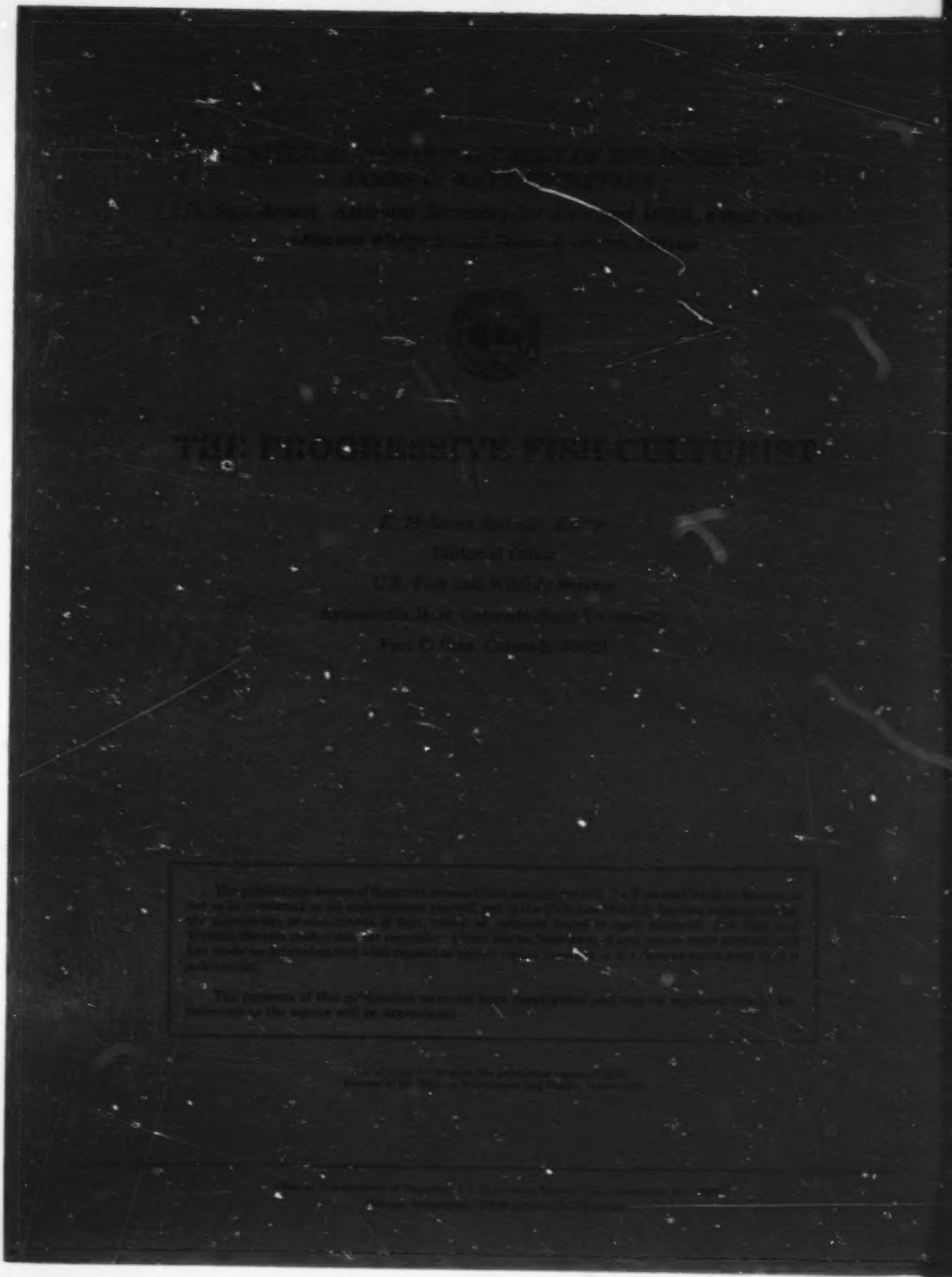

THE

Progressive Fish-Culturist

A Quarterly for Fishery Biologists and Fish-Culturists

Volume 44 April 1982 Number 2

CONTENTS

Temperature Requirements for the Survival and early Development of the Anadromous Alewife. *R. L. Kellogg* 63

Spawning Success of Reciprocal Hybrid Pairings between Blue and Channel Catfishes with and without Hormone Injection. *D. Tave* and *R. O. Smitherman* 73

Mobile Surgical Table with Automatic Solenoid System for Fishery Applications. *J. M. McKim* and *R. F. Syrett* 75

Toxicity of Rotenone to Selected Aquatic Invertebrates and Frog Larvae *J. H. Chandler, Jr.*, and *L. L. Marking* 78

Simple, Versatile Microscopic Stage for the Identification of Pinned Adult Insects. *J. H. Chandler, Jr.* 80

Avoidance Response of Rainbow Trout to Phenol. *G. M. DeGraeve* 82

Comparison of Cleithra and Scales for Age and Growth Analysis of Yellow Perch. *D. N. Schmitt* and *W. A. Hubert* 87

Aeration of Water Supplies for Fish Culture in Flowing Water. *R. W. Soderberg* 89

Enhancement of Growth of Common Carp by Injection of a Homogenate of Carp Pituitary Glands. *I. A. Adelman* 94

Effects of Wescodyne and Malachite Green on Parasitic Ciliates of Juvenile American Lobsters. A. D. Boghen 97

Technique for Photographing Small Fish. *T. M. Baugh* 99

Use of Ozone in a Water Reuse System for Salmonids. *R. C. Williams, S. G. Hughes,* and *G. L. Rumsey* 102

Sex-weight Relations in Blue, Channel, and White Catfishes: Implications for Brood Stock Selection. *M. J. Brooks, R. O. Smitherman, J. A. Chappell,* and *R. A. Dunham* 105

Device for Injecting Preservative into the Stomach of Fish. *R. L. Emmett, W. D. Muir,* and *R. D. Pettit* 107

Artificial Turf as a Substrate for Incubating Lake Trout Eggs on a Reef in Lake Superior. *B. L. Swanson* 109

Water-stable Diet for Aquatic Invertebrates. *J. J. Rach* 111

Two Economical Photoperiod Controls for Laboratory Studies.
F. H. Everest and *J. Rodgers* 113

Saturator System for Generating Toxic Water Solutions for Aquatic Bioassays.
G. L. Phipps, G. W. Holcombe, and *J. T. Fiandt* 115

Temperature Requirements for the Survival and Early Development of the Anadromous Alewife

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ABSTRACT: Studies were conducted to determine the effects of temperature on the hatching success of eggs of the alewife (*Alosa pseudoharengus*) and on the growth, survival, upper tolerance limit, and temperature preference of alewife larvae from the Hudson River. When eggs were incubated at seven constant temperatures ranging from 12.7 to 29.7° C, maximum hatching success occurred at 20.8° C. Hatching success fell significantly ($P < 0.05$) below the maximum when eggs were incubated at 26.7–26.8° C, and no hatch occurred at 29.7° C. The average time to median hatch varied inversely with temperature, ranging from 7.4 days at 12.7° C to 3 days at 23.8–23.9° C and 26.7–26.8° C. The upper tolerance limit of yolk-sac larvae acclimated to 14–15° C and exposed to elevated temperatures for 24 h was about 31° C. At seven constant temperatures ranging from 12.9 to 29.1° C, average daily rates of larval weight gain during the first 12 days after hatch increased with each higher temperature to a maximum of 83.8 μg/day (dry weight) at the highest rearing temperature (29.1° C). The maximum net biomass gain occurred at 26.4° C. The final preferendum of young alewives was estimated to be 26.3° C by monitoring their distribution in a thermal gradient during a 33-h period.

The alewife (*Alosa pseudoharengus*) is indigenous to the Atlantic coastal drainage of North America from Newfoundland to South Carolina and has become established in many landlocked lakes and impoundments throughout North America (Mansuetti and Hardy 1967; Scott and Crossman 1973). Marine populations of alewife spend most of their lives at sea, and use freshwater streams, lakes, and large rivers as spawning and nursery areas. Adult alewives enter fresh water during the early spring to spawn in shallow areas along the water's edge or in small tributaries. The young remain in the tributaries and estuaries throughout the spring and early summer, and then migrate to the sea as water cools in the early fall (Bigelow and Schroeder 1953).

The objective of the present study was to determine the temperature requirements for alewife eggs and larvae while they inhabit freshwater and estuarine nursery areas.

Materials and Methods

Thermal tolerance experiments were conducted on eggs and yolk-sac larvae, larval growth and survival were determined for the first 12 days after hatch at several constant temperatures, and the temperature preference of post yolk-sac larvae was determined. Experiments were conducted during April–May 1976 and May–June 1977 at a bioassay laboratory located at the Roseton Generating Station on the Hudson River (river mile 67). Hatching success tests were conducted in 1976 and 1977; tests on larval growth and survival and temperature preference experiments were conducted in 1977. The laboratory was supplied with a constant flow of clarified Hudson River water treated with biodegradable polymers (American Cyanamid Magnifloc 515C and 527C) before filtration. After clarification and filtration, river water was heated with steam or cooled with chilled glycol by heat exchangers to supply the laboratory with hot and cold water. Conductivity of the water during the study ranged from 100 to 226 μmhos, pH from 7.0 to 8.3, and dissolved oxygen from 7.6 to 10.6 mg/L.

Thermostatically controlled valves were used to mix hot and cold river water to produce a continuous flow of water adjusted to preset temperatures. Temperature fluctuations were generally restricted to within about 1 Celsius degree. For hatching success tests conducted during the spring of 1976, incubation temperatures were recorded to the nearest 0.1 Celsius degree with calibrated mercury thermometers 4–8 times daily, and the mean incubation temperature was determined as the average of all temperatures recorded during the period of incubation. For the tests conducted in 1977, temperatures were recorded by using a computerized data acquisition system that monitored the output of thermister probes (accurate to within 0.25 Celsius degree) in each water bath once per hour throughout

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the test. The hourly temperature data were recorded on magnetic cassette tapes and then analyzed by computer for mean and standard deviation. Mean temperatures for the 1977 tests were determined as the average of all temperatures recorded during the interval of exposure.

Hatching Success

Alewife eggs were obtained by artificially spawning adults collected from the Hudson River and its tributaries. Artificial propagation was accomplished by fertilizing eggs stripped from ripe females with milt from ripe males according to the dry method described by Davis (1967). Eggs were incubated in polyvinyl chloride (PVC) "incubation containers" that were 7.6 cm in diameter and 9.5 cm deep, with 0.5-mm screened bottom and sides. Incubation containers with eggs were placed in flow-through water baths adjusted to the desired incubation temperature. Incubation containers were supported above the bottom of the water bath to allow water to flow under the eggs to prevent stagnation within the containers.

The effect of temperature on the hatching success of alewife eggs was determined for eggs from two females during the spring of 1976 and for eggs from four females during the spring of 1977. Eggs from each female were placed in separate incubation containers and transferred to a series of flow-through water baths adjusted to constant temperatures ranging from 15 to 27° C in 1976 and from 13 to 30° C in 1977. Sample sizes tested per test temperature were 100 eggs in 1976 and 200 eggs in 1977. Selection of dead eggs, as indicated by opacity, was avoided. In 1976, the eggs were fertilized in the laboratory at 16° C and transferred to the test temperatures within 2–4 h after fertilization; in 1977, the eggs were fertilized in the field at 13–14° C and transferred to the test temperatures within 8–12 h after fertilization. The eggs were maintained at the spawning temperatures until transfer to the higher test temperatures. The exact stage of egg development at the time of transfer was not observed.

Dead eggs were removed and counted daily. Containers were examined for newly hatched larvae every 10–12 h. After initial hatching, observations were made every 2–4 h. Upon hatching, larvae were segregated into three groups (normal, dead, and deformed) and then counted. Larvae with marked curvature of the spine or other gross developmental abnormalities were classified as deformed.

Hatching success data were converted to normalized percentage values to account for variations in the percentage viability of eggs from different females and transformed to angular degrees with the arcsin transformation for statistical tests (Sokal and Rohlf 1969). Data were normalized by setting the maximum success of hatching for any replicate to 100% and setting all lesser degrees of success within the replicate as a percentage of that maximum (McCormick et al. 1977). The Student-Newman-Keuls multiple range test (for unequal sample sizes) was used to determine significant differences in hatching success between incubation temperatures. Hatching success was evaluated in terms of normal hatch only (i.e., deformed larvae were included with the dead count).

Thermal Tolerance of Larvae

The tolerance of newly hatched larvae (less than 1 day old) exposed to elevated temperatures for 24 h was determined for larvae hatched from eggs incubated at 14–15° C. Larvae were tested in 0.25-mm-mesh screen-walled containers 5 cm in diameter and 5 cm deep with shallow (1 cm deep) plastic bottoms to prevent larvae from being exposed to the air during transfer of the cups from the holding facilities to the aquaria. After placing 15–30 larvae in each container, tests were conducted by transferring each container to an aquarium adjusted to the desired temperature. Temperature fluctuations within the testing aquaria were maintained within ± 0.3 Celsius degree during the tests. At the end of the 24-h exposure, the number of live and dead larvae were recorded. Live larvae exhibiting loss of equilibrium or physical damage (deterioration of tissues, predominantly in the tail region) were counted with the dead larvae for the mortality assessment. Control larvae were treated in the same manner as experimental larvae, except that the test and acclimation temperatures were the same.

Five series of tests consisting of a control and three test temperatures were completed on separate larval stocks. Control mortality ranged from 0 to 9% among the five tests; overall control mortality averaged 4.2%. Thermal tolerance results were not corrected for control mortality.

Larval Growth and Survival

Rates of growth (dry weight), mortality, and net biomass change were determined for groups of larvae reared at 7 constant temperatures ranging from 12.9 to 29.1° C for 12 days after hatch Larvae reared at 12.9, 15.2, 18.2, and 20.8° C were obtained from eggs incubated at those temperatures. Larvae used for rearing at 23.7, 26.4, and 29.1° C were obtained from eggs incubated at 21° C and were acclimated gradually to the higher rearing temperatures at a rate of 1.0–1.5 Celsius degrees per hour.

Larvae were tested in three 8-L glass rearing tanks at each temperature. All tanks were covered with black plastic on the outside and immersed in water baths adjusted to the desired temperatures. Two tanks were supplied with water adjusted to a salinity of 1.0–1.3°/₀₀; the third tank was supplied with fresh water (less than 0.1°/₀₀ salinity).

Initially, 500 newly hatched larvae were counted

and placed in each tank. All tests were conducted under static conditions to prevent live food items from being flushed from the aquaria. About 25% of the water in each tank was changed daily and light aeration was supplied to prevent stagnation. The tanks were cleaned daily to remove accumulations of uneaten food, dead larvae, and debris. Dissolved oxygen levels remained near 90–100% saturation in all tanks throughout the test (concentrations ranged from 7.2 to 9.8 mg/L).

Larvae were fed a variety of live food organisms twice daily, beginning on the second day after hatch. Rotifers, copepod adults and nauplii, cladocerans, and algae were collected from the river with a 0.07-mm-mesh plankton net and fed to larvae. Ciliated protozoa (*Paramecium multimicronucleatum*) were filtered from cultures maintained in the laboratory and introduced into each tank with the wild zooplankton. Brine shrimp nauplii (*Artemia*) were fed to fish beginning on the 8th day of the experiment. This feeding regime provided a constant supply of live food of various sizes throughout the test.

At the same time that the 500 newly hatched larvae were placed in each rearing tank, subsamples of 50–150 additional larvae were removed from each of the 4 incubation temperatures and preserved in 5% formalin for later weight determinations. Larvae remaining in each tank at the end of the experiment were preserved similarly before weighing. Dry weights of the initial and final samples were determined by drying preserved specimens in an oven for 24 h at 68°C and weighing to the nearest 0.01 mg. Average dry weights were calculated from the total dry weight and the number of larvae weighed. In addition, total lengths (to the nearest 0.1 mm) were determined on subsamples of 5 larvae that were removed from each tank on the 6th day of the test, 10 larvae from each of the 4 initial larval stocks, and 10 larvae from each rearing tank at the end of the test.

Average daily rates of growth, mortality, and net biomass gain were calculated according to the following equations:

$$\text{Daily growth rate} = \frac{\Delta \text{ weight}}{\Delta \text{ time}} = \frac{\bar{w}_2 - \bar{w}_1}{\Delta t}$$

$$\text{Daily mortality rate} = \frac{\Delta \text{ numbers}}{\Delta \text{ time}} = \frac{n_1 - n_2}{\Delta t}$$

$$\text{Daily net biomass gain per rearing tank} = \frac{\Delta \text{ biomass}}{\Delta \text{ time}} = \frac{n_2\bar{w}_2 - n_1\bar{w}_1}{\Delta t}$$

where $\bar{w}_1$ = average weight (mg) of larvae at beginning of test (0.04 mg), $\bar{w}_2$ = average weight (mg) of larvae at end of test, n_1 = number of larvae (500) at beginning of test, n_2 = number of larvae at end of test, and Δt = the length of the test (12 days).

Temperature Preference

The temperature preference of young alewives was determined by monitoring the position of the fish in a vertical thermal gradient over a 33-h period. Two replicates were conducted simultaneously. The fish used for the experiments were reared at the laboratory in a 190-L tank supplied with an intermittent flow of unfiltered Hudson River water. The fish tested were about 40 days old and ranged from 19 to 31 mm total length. Most of the young alewives were in an advanced postlarval stage of development, but some were early juveniles.

Thermal preference experiments were conducted in two vertical gradient tanks modified from the design of Gift and Wyllie (1975). Each vertical preference tank consisted of a 2.1-m fiberglass tank, 0.9 m in diameter, with an observation window 0.3 x 2.1 m. A mixture of hot and ambient water entered the top of each tank at a rate of 3.8–7.6 L/min through a head box and exited through a circular drain at the bottom of the tank. The warm water was cooled as it traveled down the tank by a 45.7-m coil containing chilled ethylene glycol. Twelve temperature probes spaced evenly from the top to the bottom of the tank were used to record the vertical gradient. Observations were made from a dark enclosure constructed around the observation window. The observation window was covered with a sheet of plastic that was silvered on one side to enable undetected observation of the test fish. Light intensity at the surface of the tank was about 947 lux (88 ft-c) during the observation periods.

Because temperature gradients were established by cooling heated water, rather than by the more conventional method of heating cooled water, the potential for gas supersaturation in the warmer zones of the gradient was reduced. The heated influent water for all tanks was aerated in the head boxes with air stones to further reduce gas supersaturation. The percentage saturation of dissolved oxygen typically ranged from 60 to 100% at the cold end of the tanks and from 80 to 110% at the hot end.

Samples of 7 and 15 fish were tested during the two temperature preference experiments. The fish were allowed to adjust to the test tanks at ambient river water temperatures (20°C) for 24 h before testing. The thermal gradient was then initiated by mixing hot water with water at the ambient temperature in the influent head box and allowing chilled glycol to circulate through the cooling coils. The desired temperature gradient was established within about 2-4 h and observations were then made by recording the position of each fish along with a temperature record of the thermal gradient. Three observations taken at 5-min intervals constituted an observation unit. Seventeen observation units were completed during the daylight hours of the 33-h test. The gradient was shifted

occasionally during the tests to eliminate the possibility of spatial preference being misinterpreted as temperature preference. Temperature extremes within the thermal gradient were maintained at 2–3 Celsius degrees below ambient river water temperatures and 3–7 degrees above the highest temperatures at which fish were observed.

The mean, standard deviation, mode, and range of preferred temperatures were determined for each replicate and for the two replicates combined. In both replicates, the fish moved to their final preferred temperature almost immediately after the gradient was established and remained at or near that temperature for the duration of the test. Thus it was not necessary to omit the early observations from the calculations of the mean and standard deviations, as the transition time from the acclimation temperature to the final preferred temperature was short. The range of preferred temperatures included those temperatures at which fish were most frequently observed, and was visually approximated on the basis of complete frequency distributions.

Results and Discussion

Hatching Success

The mean percentage hatch of all six replicates (after normalization) indicated that the optimum temperature for hatch was 20.8°C. However, considerable variation in hatching success was observed among eggs from different females (Table 1). Although the percentage hatch for the eggs of most of the females was lower at 26.7–26.8°C than at the cooler temperatures, the highest percentage hatch of eggs from female no. 4 occurred at 26.7°C. In contrast, the highest percentage hatch for the eggs of two other females (nos. 2 and 3) was observed at the lowest temperatures tested (12.7 and 15.0°C, respectively). The highest percentage hatch for the remaining three groups of eggs occurred at 20.8°C (the overall optimum). No hatch occurred at 29.7°C. Results of the Student-Newman-Keuls multiple range test indicated that the percent hatch at 26.7–26.8°C was significantly lower ($\alpha = 0.05$) than at the optimum temperature of 20.8°C, and the zero hatch at 29.7°C differed significantly from all other groups. No other comparisons resulted in a significant difference.

Edsall (1970) observed similar temperature requirements for survival of eggs from Lake Michigan alewives incubated at temperatures ranging from 5.6 to 30.6°C. He reported that hatching occurred over a range of temperatures from 6.9 to 29.4°C, but that the highest percentage hatch of alewife eggs generally occurred at temperatures above 10°C and below 26.7°C. The optimum temperature for hatch of eggs from Lake Michigan alewives was about 17.8°C, which was lower than the optimum temperature for hatch observed during the present study (20.8°C).

Incubation temperature also had a pronounced effect on the time required for development and hatch of alewife eggs. The average time to median hatch progressively decreased from 7.4 days at 12.7°C to 3 days at 23.8–23.9°C (Fig. 1). Further decreases in incubation time were not observed at temperatures higher than 23.9°C. Times to median hatch at 20.8°C,

Table 1. Percentage hatch of normal larvae from alewife eggs incubated at constant temperatures.[a]

Mean incubation temperature (°C ± 1 SD)	Normal Hatch (%)[b]						Mean normal hatch (%) for 1976-77 combined results[c]
	1976 Results		1977 Results				
	Female No. 1	Female No. 2	Female No. 3	Female No. 4	Female No. 5	Female No. 6	
12.7 ± 0.5	—	—	77.0	56.0	61.0	60.0	71.5
15.0 ± 0.2	54.5	41.5	—	—	—	—	75.8
18.1 ± 0.2	67.5	37.0	58.0	57.0	60.0	63.0	68.1
20.8 ± 0.3	70.5	39.0	—	—	—	—	83.2
20.9 ± 0.4	—	—	74.0	68.0	72.0	70.0	
23.8 ± 0.4	61.5	38.5	—	—	—	—	67.9
23.9 ± 0.1	—	—	58.0	68.0	66.0	48.0	
26.7 ± 0.2	—	—	16.0	73.0	25.0	21.0	51.4
26.8 ± 0.6	51.5	33.0	—	—	—	—	
29.7 ± 0.0	—	—	0.0	0.0	0.0	0.0	0.0

[a] In 1976, eggs were 2-4 h old when incubated. In 1977, eggs from females 3, 4, 5, and 6 were incubated 12, 10, 9, and 8 h after fertilization, respectively.
[b] Raw data, before normalization and transformation to arcsin $\sqrt{\%}$ for statistical tests.
[c] Percentage mean hatch after normalization and arcsin transformation.

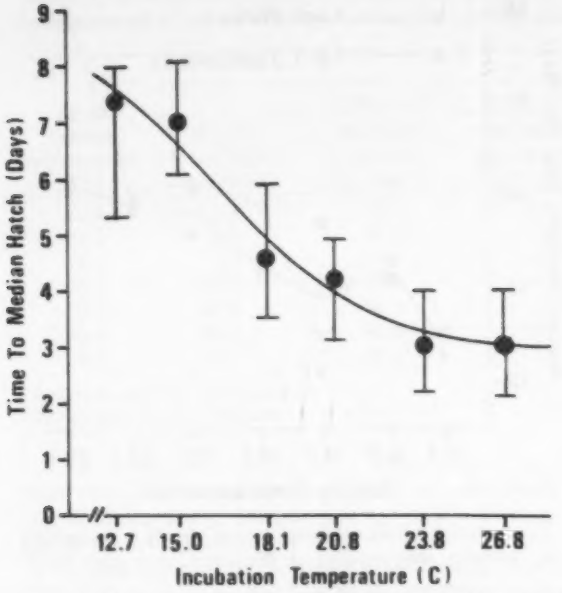

Fig. 1. Average time to median hatch (•) and range of hatching times (I) for alewife eggs incubated at six constant temperatures. The curve was fitted by inspection.

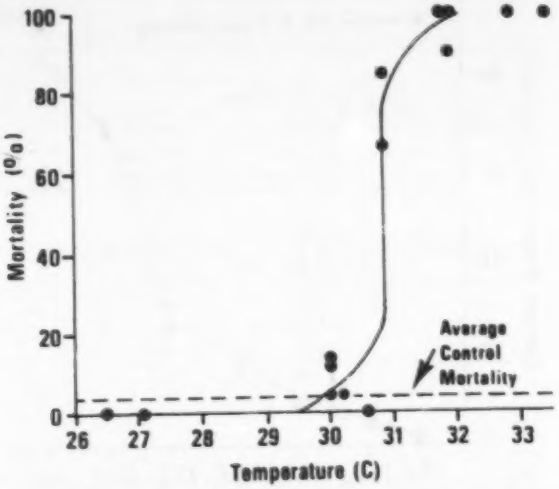

Fig. 2. Thermal tolerance of alewife yolk-sac larvae (less than 1 day old) exposed to elevated temperatures for 24 h. Larvae were hatched from eggs incubated at 14–15° C before exposure. The mortality curve was fitted by inspection.

the optimum temperature for normal hatch, averaged 4.1 days. Incubation times of 6 days at 15.5° C (Bigelow and Welsh 1925), 3–5 days at 20° C (Mansuetti and Hardy 1967), and 2–4 days at 22° C (Belding 1921) reported for alewife eggs from other marine stocks correspond with the incubation times found in this study. Incubation times reported by Edsall (1970) for Lake Michigan alewives are also in general agreement with results reported here, except that Edsall observed that incubation time continued to decrease to a minimum of 2.1 days at 28.9° C.

Thermal Tolerance of Larvae

Larval mortality resulting from 24-h exposures to elevated temperatures occurred as temperatures exceeded 30° C, and was 100% at temperatures greater than 32–33° C (Fig. 2). The upper TL50 (i.e., the temperature resulting in 50% survival) for these alewife yolk-sac larvae acclimated to 14–15° C was about 31° C.

Thermal tolerance for alewife yolk-sac larvae was in close agreement with the upper thermal tolerance limits reported for older young-of-the-year alewives, in spite of differences in age and life stage. Stanley and Colby (1971) reported that significant mortality of young Lake Michigan alewives acclimated to 16.2° C occurred at 30–31° C when the fish were subjected to a gradual temperature increase at a rate of about 2.5 Celsius degrees per day. Dorfman and Westman (1970) observed initial mortalities of young-of-the-year alewives acclimated to 18.9–20.6° C when they were exposed to 30–32° C. Their studies exposed the fish to higher temperatures at rates ranging from a maximum of 6.7 to a minimum of 0.6 Celsius degrees per day; the slower heating rates were employed at the higher temperatures. Upper incipient lethal temperatures (4- to 7-day TL50's) for young of the year acclimated to temperatures ranging from 23 to 26° C have been reported to be 32.1° C for Lake Michigan alewives (Otto et al. 1976) and 31.7–32.6° C for Hudson River alewives (Ecological Analysts, Inc. 1978).

Larval Growth and Mortality

Average growth rates for the 12-day test period were progressively higher at successively higher temperatures from 12.9 to 29.1° C, both in fresh water and in water adjusted to a salinity of 1.0–1.3°/oo (Fig. 3; Tables 2, 3). The rate at which the average growth rate increased as rearing temperature increased was greatest for temperatures above 20.8° C, indicating that small increases in temperatures above 20.8° C can result in substantial increases in growth.

Growth rates for larvae reared in fresh and low-salinity waters were similar at temperatures ranging to 23.7° C, but at 26.4° C growth rates were considerably lower for larvae reared in fresh water than for those reared in water adjusted to a salinity of 1.0–1.3°/oo. The highest growth rates occurred at 29.1° C (the

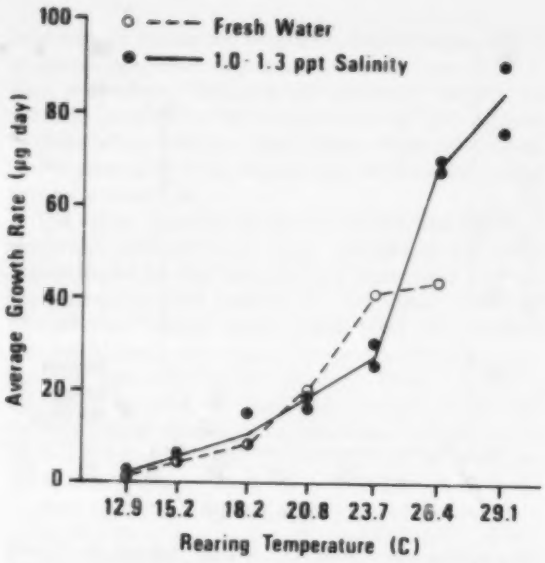

Fig. 3. Average daily growth rate (dry weight) of alewife larvae reared at 7 constant temperatures for the first 12 days after hatch.

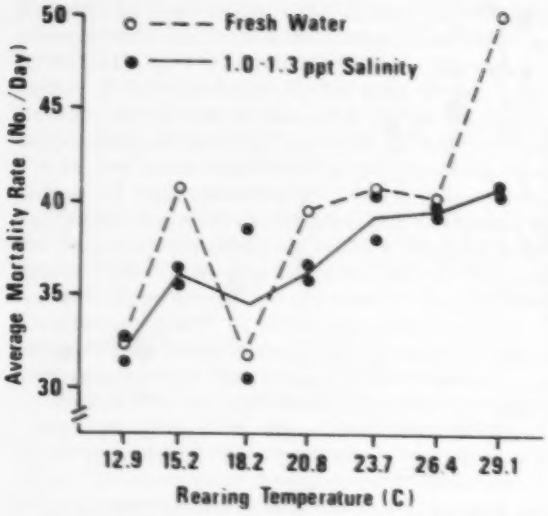

Fig. 4. Average daily mortality rate of alewife larvae reared at 7 constant temperatures for the first 12 days after hatch.

highest rearing temperature tested) in the low-salinity tanks. No larvae survived beyond 10 days at this temperature in the freshwater tank. However, mean total lengths of 5 larvae removed from each tank on the 6th day of the test indicated that the highest growth rates at that time occurred at 29.1° C in both fresh and low-salinity water (Table 4). Moreover, the average length of larvae at the end of 6 days in the freshwater tank was greater than that of larvae reared in the low-salinity tanks at 29.1° C. These data suggest that increased temperature over the range tested enhances growth in both fresh water and low-salinity water.

The average growth rate in terms of length at 20.8° C was nearly identical to that reported by Heinrich (1981) for Great Lakes alewives reared at a constant temperature of 20° C. Heinrich observed that growth rates averaged 0.52 mm/day for the first 15 days after hatching, and that the mean total length at 15 days was 11.6 mm. In the present study, mean total lengths 12 days after hatch were 11.3 and 11.5 mm for low salinity and freshwater replicates, respectively (Table 4), and the average daily growth rate was about 0.5 mm/day. (Larvae tested during the present study were initially about 1 mm longer than those tested by Heinrich, which accounts for the similarity in mean total lengths in spite of the 3-day difference in age.)

Mortality was high for all rearing conditions and was also quite variable. Most of the mortality was probably due to the unsuccessful transition to exogenous feeding, but since the daily rates were not

Table 2. Number surviving, average weight, and biomass per tank of alewife larvae after 12 days of rearing at 7 constant temperatures in fresh and saline waters. The initial number of fish per rearing tank was 500, the initial average dry weight was 0.04 mg, and the initial biomass per tank was 20 mg (dry weight).

		Salinity 1.0–1.3 °/oo						Fresh water		
		Replicate 1			Replicate 2					
Mean temperature (°C ± SD)	Mean dissolved oxygen (mg/L)	No. fish surviving	Dry wt. per fish (mg)	Biomass per tank (mg)	No. fish surviving	Dry wt. per fish (mg)	Biomass per tank (mg)	No. fish surviving	Dry wt. per fish (mg)	Biomass per tank (mg)
12.9 ± 0.6	9.8	124	0.06	7.44	108	0.05	5.40	113	0.05	5.65
15.2 ± 0.2	9.0	71	0.09	6.39	64	0.10	6.40	11	0.09	0.99
18.2 ± 0.3	8.8	133	0.14	18.62	38	0.22	8.36	120	0.14	16.80
20.8 ± 0.4	8.2	71	0.26	18.46	61	0.24	14.64	27	0.28	7.56
23.7 ± 0.3	7.8	44	0.34	14.96	16	0.40	6.40	14	0.53	7.42
26.4 ± 0.3	7.6	31	0.86	26.66	24	0.85	20.40	19	0.56	10.64
29.1 ± 0.1	7.2	14	1.12	15.68	11	0.95	10.45	0	—	0.00

Table 3. Average daily rates of growth (dry weight), mortality, and net biomass gain (dry weight) for alewife larvae reared at constant temperatures for 12 days after hatch.

Mean rearing temperature (°C)	Water: Fresh water — Growth (µg/day per fish)	Fresh water — Mortality (no/day per tank)	Fresh water — Net biomass gain (mg/day per tank)	Salinity 1.0–1.3 °/oo[a] — Growth (µg/day per fish)	Salinity 1.0–1.3 °/oo[a] — Mortality (no/day per tank)	Salinity 1.0–1.3 °/oo[a] — Net biomass gain (mg/day per tank)
12.9	0.8	32.2	-1.2	1.3	32.0	-1.1
15.2	4.2	40.8	-1.6	4.6	36.0	-1.1
18.2	8.3	31.7	-0.3	9.8	34.5	-0.5
20.8	20.0	39.4	-1.0	17.6	36.2	-0.3
23.7	40.8	40.5	-1.0	26.3	39.2	-0.8
26.4	43.3	40.1	-0.8	68.0	39.4	0.3
29.1	—[b]	50.0[b]	-2.0[b]	83.8	40.6	0.6

[a] Weighted means of two replicates.
[b] Larvae did not survive beyond the 10th day.

measured, the exact timing and cause of death could not be determined. The average mortality rates for larvae reared at a salinity of 1.0–1.3°/oo were typically lower than those for larvae reared in fresh water (Table 3; Fig. 4), suggesting that low levels of salinity might enhance larval survival, at least under laboratory conditions.

The pattern and variability of mortality appeared to be related to the developmental stage of the larvae, and probably also the extent to which the transition to exogenous feeding was complete at the end of the test. Mortality was lowest (31–33/day per tank) at the lowest temperature (12.9°C). Larvae reared at this temperature grew very little during the 12-day test (about 0.2 mm/day) and had probably just started to feed shortly before the test was terminated. The greatest variability among mortalities (31 – 41/day per tank) was observed at 15.2 and 18.2°C. These larvae were in the early postlarval stage at the end of the test. Mortality was generally higher but much less variable at 20.8, 23.7, and 26.4°C (36 – 41/day per tank) than at 15.2 and 18.2°C. Larvae at these higher temperatures were in the mid- to late-postlarval stage at the end of the test. Thus, a possible explanation for the pattern and variability of mortality is that mortality due to unsuccessful transition to exogenous food was complete at the higher temperatures (e.g., 20.8, 23.7, 26.4°C), resulting in high mortalities and relatively low variability, whereas the transition was incomplete in most aquaria at the lower temperatures, resulting in generally lower but more variable mortalities.

Mortality was greatest at the highest temperature (29.1°C). Complete mortality occurred in the fresh-water tank and larval mortality averaged 41 per day per tank in the two low-salinity replicates. It is likely that some of the high mortality at 29.1°C resulted from thermal stress, since this rearing temperature was only 2 Celsius degrees below the TL50 for alewife yolk-sac larvae.

The mortality reported by Heinrich (1981) was much lower than mortalities observed during the present study. A comparable mortality rate from Heinrich's study would have been 12 larvae per day per tank. (Heinrich reported 311 dead after 13 days in 2

Table 4. Mean total lengths (mm ± SD) of alewife larvae reared at 7 constant temperatures for 6 days and 12 days after hatch.

Rearing temperature (°C)	At start of test (40 larvae)	6 Days after hatch: Fresh water (5 larvae)	6 Days after hatch: Salinity 1.0–1.3 °/oo (10 larvae)	12 Days after hatch: Fresh water (10 larvae)	12 Days after hatch: Salinity 1.0–1.3 °/oo (20 larvae)
12.9	4.6 ± 0.3	6.0 ± 0.2	6.0 ± 0.2	7.1 ± 0.4	7.1 ± 0.5
15.2	4.6 ± 0.3	6.3 ± 0.4	6.2 ± 0.3	8.3 ± 0.4	8.6 ± 0.9
18.2	4.8 ± 0.2	6.9 ± 0.4	6.8 ± 0.5	9.9 ± 0.9	10.5 ± 1.3
20.8	5.3 ± 0.2	7.4 ± 0.9	7.0 ± 0.7	11.5 ± 0.1	11.3 ± 1.2
23.7	5.3 ± 0.2	7.4 ± 1.6	6.7 ± 0.4	12.7 ± 1.8	11.9 ± 2.2
26.4	5.3 ± 0.2	7.4 ± 1.4	7.6 ± 1.2	13.2 ± 1.5	14.7 ± 2.4
29.1	5.3 ± 0.2	8.9 ± 1.2	8.2 ± 1.2	—	14.0 ± 3.0

tanks each initially stocked with 500 larvae.) Most of the procedures used during the present study were similar to those reported by Heinrich, except for tank size. Heinrich stocked 500 larvae in a 53-L aquarium, whereas the same number of larvae were stocked in 8-L aquaria during the present study. Although other factors might also be involved, variables related directly or indirectly to tank size might account for some of the differences in the mortalities observed in the two studies.

Conclusions regarding the optimum temperatures for growth are complicated by the coincidence between the temperature at which the highest growth occurred and the temperature at which mortality was highest. To aid in determining the effects of temperature on growth and mortality simultaneously, net biomass gain values were examined (Table 3; Fig. 5). Most of these values were negative because of the relatively high mortalities at all rearing temperatures. Biomass values were also highly variable. The variability in the biomass values reflected the variability in growth as well as the larger variability in mortality. For larvae reared at a salinity of 1.0–1.3‰, biomass gain generally increased with increasing temperature to an optimum at 26.4° C and then decreased at 29.1° C. The highest biomass gain for larvae reared in fresh water occurred at 18.2° C, primarily resulting from the unusually high survival that was observed in that rearing tank. The next highest biomass gain in fresh water occurred at 26.4° C, coinciding with the highest biomass gain value observed at a salinity of 1.0–1.3‰. A reasonable estimate of the optimum temperature for growth and early development thus seems to be 26.4° C, on the basis of these results. These data further suggest that 29.1° C, the temperature at which the highest growth rate was observed, is suboptimal for young alewives. However, conclusions based solely on these biomass values are somewhat uncertain because of the high mortalities and the variability among the biomass values.

The high levels of mortality observed during the present study could create a bias in determinations of both growth rate and net biomass gain. For example, the number of larvae surviving the 12-day experiment was often less than 10% of the initial number (see Table 2). It is logical to expect that these survivors were the "hardiest" individuals among the original 500, and thus any growth rate determined on the basis of these fish may not be representative of the growth rate of an "average" individual. Although the direction of this bias is unknown, it is reasonable to assume that the hardier individuals would be able to capitalize on their hardiness and grow at rates greater than the average fish.

This potential for bias is most relevant in situations where the surviving larvae were selected on the basis of their thermal tolerance. It is possible that this kind of

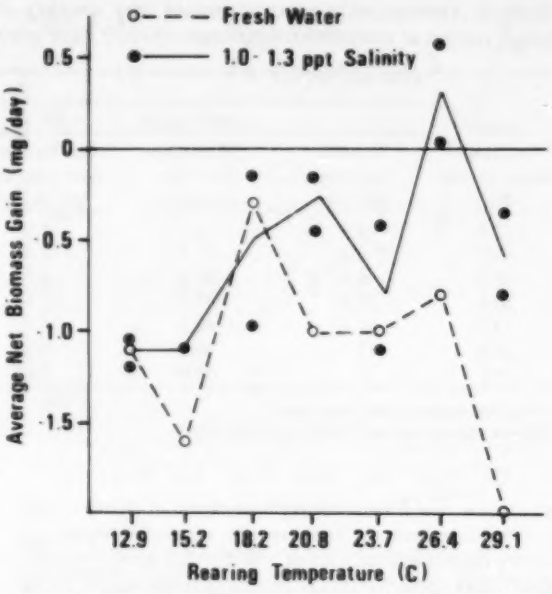

Fig. 5. Average net biomass gain per rearing tank (dry weight) for alewife larvae reared at 7 constant temperatures during the first 12 days after hatch.

selection took place at 29.1° C, since this temperature is close to the lethal limit for young alewives. Only 11 and 14 larvae survived 12 days in the two low-salinity replicates at 29.1° C. If these individuals represented only a small thermally resistant portion of the population, the growth rate of an average individual at this temperature would probably have been small since the average fish might have died or been stressed sufficiently to impede growth. Consequently, the growth rates determined for larvae reared at 29.1° C may be overestimated relative to growth rates determined at the lower temperatures. The extent of this bias at the lower temperatures would be expected to be much less serious, since the selection for the hardier individuals would not be expected to be based on thermal resistance.

Temperature Preference

The frequency distributions of the two replicate temperature preference tests were nearly identical (Table 5). The mean preferred temperatures for the two replicates differed by only 0.3 Celsius degree and the modal preferred temperatures differed by only 1 Celsius degree. The mean preferred temperature for the combined replicates was 26.3° C (standard deviation, 2.0° C). The modal preferred temperature, 26–27° C, coincided closely with the mean preferred temperature; results were considered bimodal because there was a difference of less than 0.2 percentage point between the percentages of occurrence at 26 and 27° C

(see Fig. 6). The range of preferred temperatures was estimated to extend from 25 to 28° C; 75% of the temperature preference observations occurred within this temperature range.

The mean preferred temperature for the combined replicates was taken as the best estimate of the final preferendum, defined by Fry (1947) as the "temperature around which all individuals (of a given species) will ultimately congregate, regardless of their thermal experience before being placed in the gradient" (see also Reynolds and Casterlin 1979). Both the mean and the modal preferred temperatures are commonly used to estimate the final preferendum (Richards et al. 1977). Since the fish exhibited no trend of increasing or decreasing temperature preference throughout the 33-h test period, the mean preferred temperature appears to be a reliable estimate of the final preferendum in the present study. The suitability of the mean as an estimate of the final preferendum is further supported by the nearly normal frequency distribution of preferred temperatures (Fig. 6).

The correspondence between the final preferendum (26.3° C) and the optimum temperature for growth and early development (26.4° C) is striking. Brett (1971), Coutant (1972), Beitinger and Fitzpatrick (1979), and others have reported that optimum temperatures for a wide variety of physiological functions often coincide at a temperature that is characteristic for a species (or life stage), and that this overall optimum temperature, in turn, often coincides with the final preferendum. The results of the growth experiments and the temperature preference experiments together indicate that the optimum temperature for larval growth and development of the alewife is about 26° C.

It is also noteworthy that alewife larvae avoided temperatures that were suboptimal or lethal for prolonged exposures. Only 11% of the temperature preference observations occurred at 29° C and above. About 3% of the temperature preference observations were at 30° C or above, and no fish were observed above 32° C, even though temperatures as high as 35–36° C were frequently available in the temperature gradient. During the present study, temperatures in this range were demonstrated to be suboptimal (29° C) or lethal for prolonged exposures (30–31° C). These data suggest that in the natural setting alewife larvae would be expected to avoid potentially lethal or suboptimal thermal zones whenever possible.

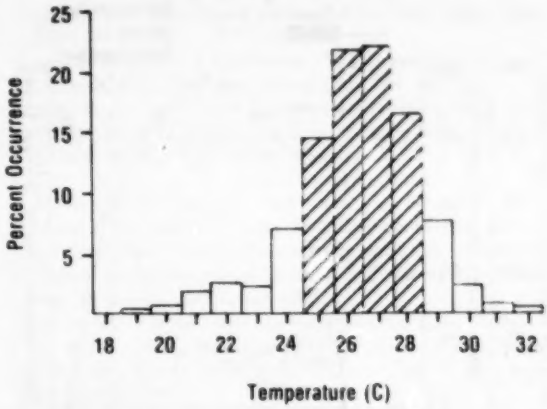

Fig. 6. Temperature preference histogram for young alewives tested in a thermal gradient over a 33-h period (combined results from two replicates). The range of preferred temperatures is indicated by the shaded portion of the histogram.

Comparison of Temperature Requirements with Spawning Temperatures

Temperature requirements determined during the present study are summarized and compared with spawning temperatures reported for anadromous alewives in Fig. 7. Peak spawning typically occurs at 13–16° C (Tyus 1974; Richkus 1974; Cianci 1965), although some spawning has been reported at temperatures as low as 10° C (Cianci 1965) and as high as 20° C (Smith 1971; Kissil 1974). In the present study, the highest temperature at which normal hatch was not substantially reduced by temperature was about 24° C, which exceeds peak spawning temperatures by 8–11 Celsius degrees. Newly hatched larvae survived 24-h

Table 5. Results of temperature preference experiments conducted with young alewives.

Replicate	No. of fish tested	Mean total length (mm)	Sample[a] size	Preferred temperatures (° C) Mean	Standard deviation	Mode	Range	Observations within range (%)
1	7	24	263	26.6	2.3	26	26–29	76
2	15	23	776	26.3	1.9	27	25–28	77
Combined	22	23	1,039	26.3	2.0	26,27[b]	25–28	75

[a] Number of fish observed at each observation summed over all the observations.
[b] Less than 0.2% difference between percentages of occurrence at these two temperatures.

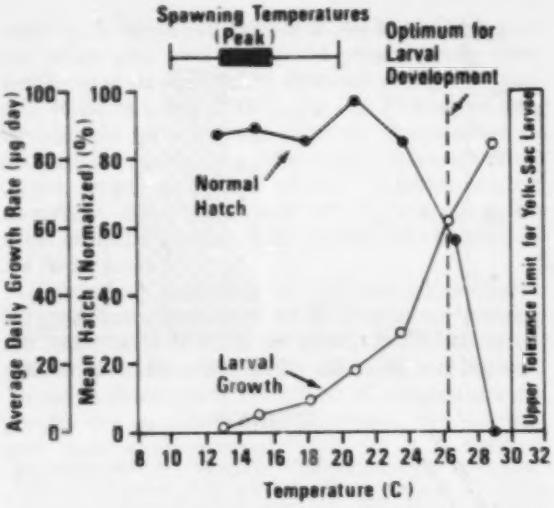

Fig. 7. Summary of temperature requirements for the survival and early development of anadromous alewife. Spawning temperatures were obtained from the literature (see text); temperature requirements were determined during this study. Larval growth values are for the combined results of freshwater and low-salinity water replicates, and the optimum temperature for larval development was based on both net biomass gain values and the final preferendum of postlarvae.

exposures to temperatures up to about 30° C, which exceeds peak spawning temperatures by 15–18 Celsius degrees. The optimum temperature for larval growth and development was estimated to be about 26° C on the basis of both growth and temperature preference experiments. This optimum growth temperature exceeds peak spawning temperatures by about 10–13 Celsius degrees. Consequently, it is not likely that the survival and early development of young alewives would be threatened by rapid warming trends following spawning or by moderate thermal discharges; in fact, temperature elevations above normal following spawning and hatch would probably be beneficial to alewife populations.

Acknowledgments

This research was part of an investigation on the effects of thermal discharges from Hudson River power plants on the Hudson River estuary and was jointly supported by Central Hudson Gas and Electric Corporation, Consolidated Edison Company of New York, Inc., and Orange and Rockland Utilities, Inc. I thank Thomas Huggins of Central Hudson Gas and Electric Corporation and Jay Hutchison of Orange and Rockland Utilities, Inc., for their assistance and cooperation during the investigation, and employees of Ecological Analysts, Inc., for their help during all phases of this study.

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Accepted 27 October 1981

Spawning Success of Reciprocal Hybrid Pairings Between Blue and Channel Catfishes With and Without Hormone Injection

Hybridization between blue catfish (*Ictalurus furcatus*) and channel catfish (*I. punctatus*) has increased yield (Guidice 1966; Yant et al. 1975; Chappell 1979), dressing percentage, feeding vigor, percent marketable fish (Chappell 1979), and catchability (Tave et al. 1981) over those of the parent species. Unfortunately, reliable spawning techniques to produce these hybrids have not been developed; before they can be recommended to farmers, hybrid production techniques must be quantified or the commercial use of these fish will not be practical. The object of the present study was to determine which hybrid is easier to produce and to determine whether hormone injection increases spawning success.

Nine pairings in each of four groups — channel catfish female x channel catfish male, blue catfish female x blue catfish male, channel catfish female x blue catfish male, and blue catfish female x channel catfish male — were made on 18 May 1977. All pairs were placed in individual spawning pens in a single earthen pond at the Marion (Alabama) Fish Hatchery. Fish used in the present study ranged from 1.9 to 5.4 kg (average 3.9 kg). Fish in each pair were of similar size to prevent injury during courtship behavior.

Five randomly chosen females from each group were injected intraperitoneally with 1,100 I.U. human chorionic gonadotropin (HCG) per kilogram of body weight on 18 May; the remaining four in each group served as controls. If an injected female did not spawn after the first injection she was reinjected up to three times (28 May and 2 and 9 June) before the experiment was terminated. Water temperature ranged from 22 to 23° C.

Spawning pens were checked every 2 days, and egg masses with eyed eggs were removed and placed in troughs with paddlewheel aerators. Spawning success in the parental lines were excellent. In each parental group one fish died as a result of fighting and one fish jumped from its spawning pen into the pond. If the pairings where the fish escaped are ignored, seven of eight pairs spawned (88%). Injected fish did not spawn earlier than uninjected fish. All injected fish spawned after one injection.

Spawning success in the hybrid pairings was far less than in the parental groups. No blue catfish female x channel catfish male pair spawned. Two of the five

injected channel catfish female x blue catfish male pairs spawned after one injection (40%). The other females were injected a total of four times, but none spawned. No uninjected female spawned.

Hatching success from all egg masses was similar and estimated at 90%. Total fry production was 88,000 channel catfish, 112,000 blue catfish, and 26,000 channel catfish female x blue catfish male hybrids. Fry production per kilogram of female was 3,120 for channel catfish, 4,730 for blue catfish, and 4,262 for channel catfish female x blue catfish male hybrid pairings.

These data suggest that the channel catfish female x blue catfish male hybrid is easier to produce than the reciprocal hybrid. This is fortunate since previous studies have shown that the channel catfish female x blue catfish male hybrid is superior to its reciprocal under normal culture conditions. It is more uniform in total length (Brooks 1977), grows faster in yield trials, is more seinable, exhibits greater feeding vigor, has a better food conversion, has a superior dressing percentage (Chappell 1979), and is more catchable (Tave et al. 1981).

There appears to be an ethological block that inhibits hybridization between these two species. Both species spawned synchronously, suggesting that there is no environmental block. There appears to be no gametic or developmental block inhibiting hybridization, since hybrids were produced. Because hybrids were produced following hormone injection, it appears that the key to hybrid catfish production lies in behavioral manipulation.

The behavioral inhibition to hybridization was partly circumvented through HCG injection in the channel catfish female x blue catfish male hybrid pairing. Spawning success in this hybrid pairing might be improved by increasing hormone dosage, increasing the frequency of hormone injection, through the injection of fish pituitaries, or by hormone injection of the male. Additionally, the spawning behavior of the two species should be studied to determine differences and similarities. When these characteristics are known, behavioral manipulation other than hormone injection could be used to produce hybrid catfish.

Acknowledgments

We thank Joseph H. Addison, Jesse A. Chappell, Andrew S. McGinty, and Jerry L. Moss for assistance in the field and Katherine B. Tave for critical review of the manuscript.

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Accepted 20 November 1981

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Mobile Surgical Table with Automatic Solenoid System for Fishery Applications

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ABSTRACT: A self-contained, mobile surgical table for fishery applications is described. An automatic solenoid switching system provides for controlled delivery of anesthetic and anesthetic-free water.

Surgical tables are used extensively for studies on fish physiology and toxicology. They are particularly valuable for preparing fish for in-depth pharmacodynamic studies, which involve analyses of the movement of toxics across biological membranes and the in vivo sampling of blood, bile, urine, and feces (Schmidt and Weber 1973; Hunn and Allen 1974; Gingerich et al. 1978; Kobayashi and Nakamura 1979; Meyerhoff 1980; Schmidt and Kimerle 1981; McKim and Goeden, in press).

New analytical techniques, which require smaller samples and yet provide increased sensitivity, have also helped to stimulate interest in pharmacodynamic studies with fish, resulting in more extensive surgical manipulations. The basic design for the first fish surgical table was described by Smith and Bell (1967), and various modifications of this version are now in use.

The present report describes a self-contained mobile surgical table for fishery applications with an automatic, solenoid valve system for controlled delivery of anesthetic and anesthetic-free water.

Design and Construction

The complete design of this surgical table is presented in two figures. Components of the table design including surgical sling, catch basin and drain, removable top, oxygen delivery system, reservoir tanks, temperature control, and cabinet construction details are shown in Fig. 1; details of the water delivery system, the surgical sling, and the solenoid switching system are shown in Fig. 2, which includes the necessary wiring diagrams for switches, pumps, and solenoids. A complete parts list is available from the authors on request.

Discussion

We describe a mobile, self-contained surgical table that provides an automatic solenoid switching system for fingertip control of anesthetic and anesthetic-free water for fishery studies.

A set of four switches controls the water delivery system and eliminates the need for manual valves and clamps. Two of the switches control the pumps in each of the reservoirs (anesthetic and nonanesthetic), and the other two operate the solenoid drain system (Figs. 1, 2) which consists of two normally closed Valcor solenoid valves, 30 psi, with 1.27-cm (0.5-in.) fittings (Valcor Engineering Corporation, Kenilworth, New Jersey). When the anesthetic pump is on, the solenoid allowing water to return from the catch basin to the anesthetic reservoir is also on; this relation is reversed when the anesthetic-free water is desired. A cleanable in-line glass-wool filter, positioned between the pump and the one-way valves in the water delivery system, removes particulate material that might interfere with the operation of the one-way valves or the needle valve. The automatic solenoid system and water delivery system described here can also easily be added to surgical tables of earlier design.

During surgery, pure oxygen is bubbled slowly into the water in both reservoirs. This is necessary to keep the fish's blood saturated with oxygen (especially in fish larger than 400 g; Smith and Bell 1967), because fish gills collapse when out of water and are therefore not efficient at obtaining oxygen (Davis and Cameron 1971). The use of pure oxygen and the maintenance of low reservoir temperatures (7–10° C) during surgery enabled us to keep rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) from 500 to 1,000 g on the surgical table for 1–2 h with no observed physiological effects.

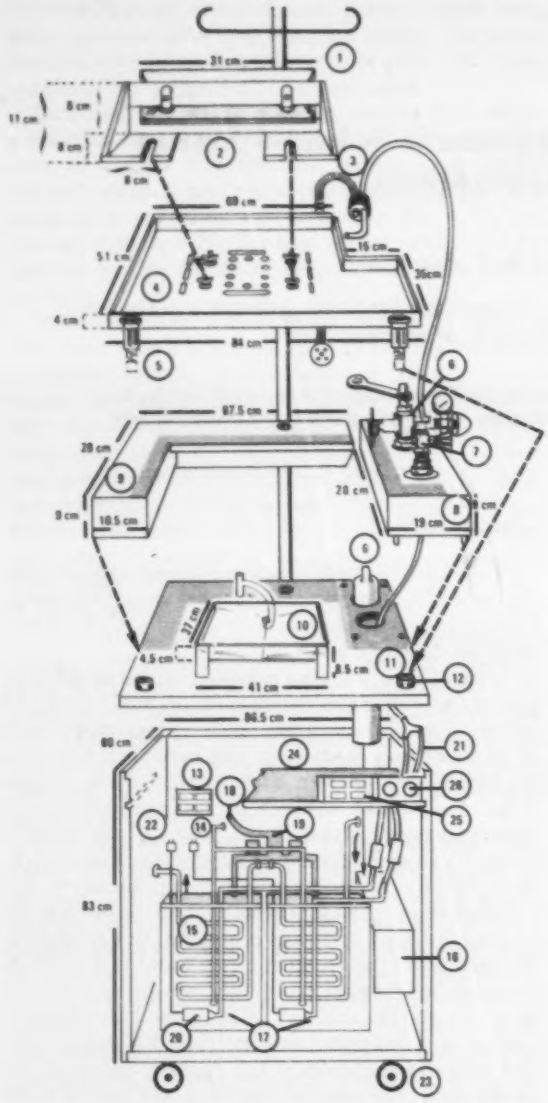

Fig. 1. Overall schematic of fish surgical table, including dimensions. 1, Flexaframe saline hanger; 2, surgical sling (see detail, Fig. 2a); 3, flexible metal arm with gill irrigator; 4, removable acrylic top with drain holes and wingnuts for surgical sling; 5, adjustable pipe leg of polyvinyl chloride (PVC); 6, oxygen tank and regulator; 7, adjustable needle valve (0.64 cm) for water delivery system; 8, removable O_2 tank cover and acrylic top support; 9, acrylic top support; 10, acrylic catch basin and drain; 11, formica cabinet top; 12, adjustable leg hole; 13, four-outlet junction box for pumps, solenoids, and switches (see Fig. 2b wiring diagram); 14, oxygen line from oxygen tank and regulator to reservoir tanks; 15, stainless steel temperature coil (0.95 x 1066.8 cm) attached to temperature mixing valve (Eastman Kodak, N.Y.); 16, shelf for oxygen tank; 17, reservoir tanks for anesthetic and anesthetic-free water (114 L); 18, drain line from catch basin; 19, automatic solenoid system (see detail, Fig. 2c); 20, submersible magnetic drive water pump; 21, water delivery system (see detail, Fig. 2d); 22, plywood cabinet; 23, casters; 24, shelf; 25, switches for pumps and solenoids (see Fig. 2b wiring diagram); 26, indicator lights (see Fig. 2b wiring diagram).

Reservoir water temperatures can be controlled over a wide range by using the temperature coils as heat exchangers in each reservoir. The proper water temperature moving through the coils is maintained by a temperature mixing valve, which mixes hot and cold water just as most shower fixtures do. This arrangement enables selection and maintenance of the desired temperature without the use of ice or special heating or cooling devices. The water source for cooling can be tap water, since the water flowing through the temperature coils has no direct contact with the reservoir water. In addition, each of the reservoirs has a capacity of 113.6 L (30 gal), which eliminates the need to continually renew the anesthetic bath during use because of loss of anesthetic potency or contamination with small amounts of blood and mucus.

The flexible metal arm to which the gill irrigator is attached enables rapid positioning of anesthetic flow through the gill slits or into the mouth without using valves or clamps. A 1.27-cm OD glass U-tube with short (7.6-cm) sections of soft rubber tubing attached is used to spread the water over the gills when the flow is directed anteriorly through the gill slits. The flow rate of anesthetic or anesthetic-free water over the gills is easily controlled by the needle valve assembly without damage to the magnetic drive pumps.

The large work area around the surgical sling makes room for necessary equipment and work space. The design of the table also allows investigators to work from both sides of the table, either for observation or as part of the surgical manipulation. The surgical sling is adjustable for fish of different sizes and can easily be removed and replaced by a larger or smaller sling, depending on fish species and size.

A jaw retractor, to hold the fish's mouth open during work inside the buccal cavity, consists of a blunt hook attached by a nylon cord to a flexaframe rod. The rod is attached at right angles to the flexaframe saline hanger. A small blunt wire hook is fastened to the removable acrylic top at a position directly under the tip of the fish's snout.

For the necessary information on anesthetics and surgical techniques available for cold-blooded vertebrates, we suggest the articles by Smith and Bell (1967), Klontz and Smith (1968), and Randall and Hoar (1971).

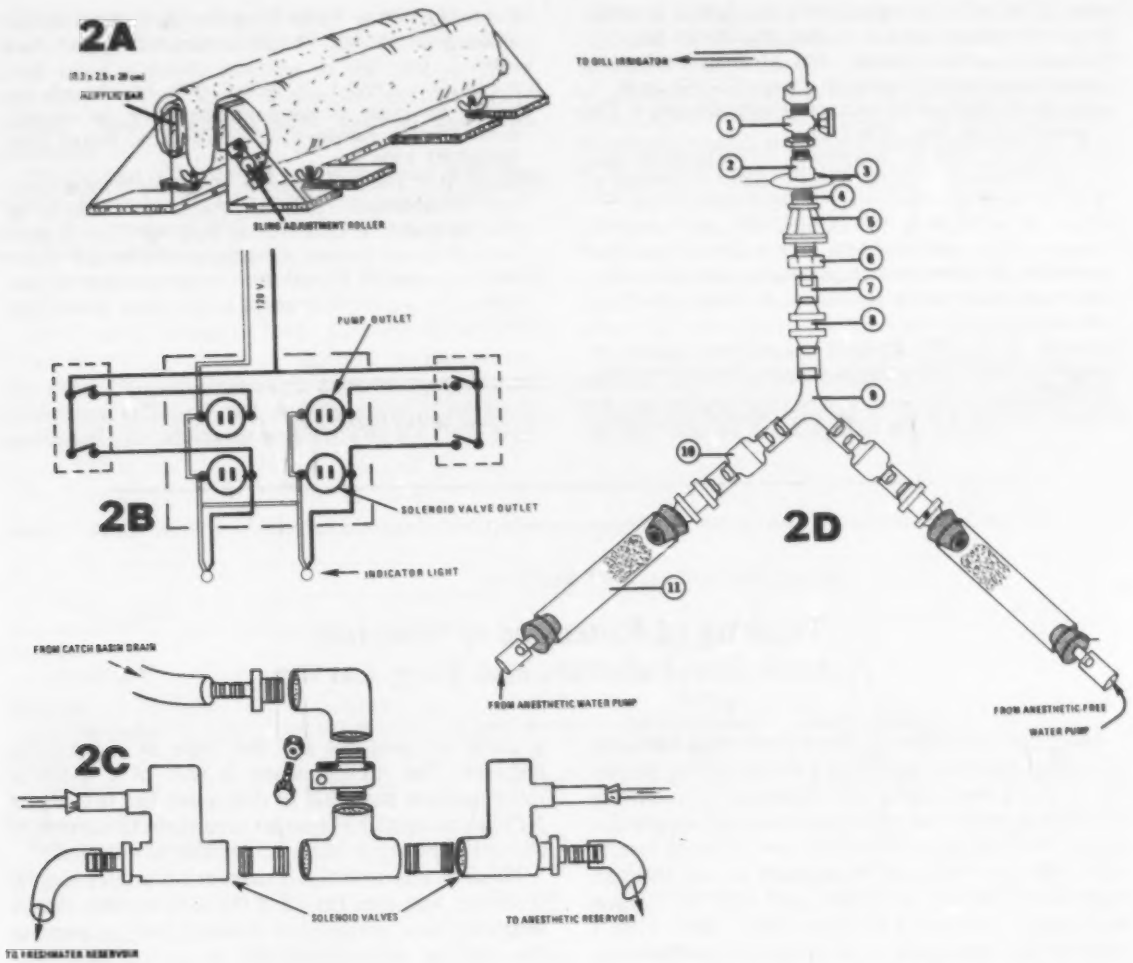

Fig. 2. (A) Detail of surgical sling. The acrylic bar is held in place by two Hoffman screw-type pinchcocks shown in Fig. 1. (B) Wiring diagram for switches, pumps and solenoids. (C) Detail of automatic solenoid system, including placement of normally closed solenoid valves and PVC pipe connections (0.127 cm; 0.05 in.). (D) Detailed schematic of water delivery system: (1) stainless steel needle valve, 0.64 cm (0.25 in.); (2) PVC reducer bushing from 0.95 cm (0.38 in.) to 0.64 cm; (3) table-top pipe flange; (4) PVC nipple, 0.95 cm; (5) PVC reducer coupling from 1.27 cm (05 in.) to 0.95 cm; (6) polyethylene tubing adapter from 1.27-cm pipe to 1.27-cm ID tubing; (7) Tygon tubing, 1.27 cm; (8) quick-connect polyethylene tubing connector, from 1.0 to 1.2 cm (0.39 to 0.47 in.); (9) Y-type polypropylene connector 0.48 cm (0.19 in.); (10) tapered-stem plastic check valves for tubing from 0.95 to 0.64 cm ID; (11) in-line glass-wool filters 2.54 cm OD, 10.2 cm long (1 x 4 in.).

Acknowledgments

We thank Lavern J. Weber, Director of the Marine Science Center, Oregon State University, Newport, Oregon, and Roger D. Meyerhoff, Eli Lilly Co., Greenfield, Indiana, for an introduction to and the use of their fish surgical table, which served as the prototype for the one described here; and Alyce Johnson for the detailed drawings of the apparatus.

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Accepted 14 December 1981

Toxicity of Rotenone to Selected Aquatic Invertebrates and Frog Larvae

More than 60 species of plants containing rotenone have been used for centuries by natives of the tropics for stunning and killing fish. Rotenone is relatively harmless to plants and mammals at normal use pattern concentrations, but is extremely toxic to many insects and other invertebrates. It appears to act through respiratory enzyme inhibition, and kills by oxygen deprivation (Metcalf and Flint 1962). Binns (1967) reported that nearly all phyla of aquatic invertebrates present in the Green River, Wyoming, were adversely affected by an application of rotenone that ranged from 2.5 to 10.0 mg/L. Almquist (1959) stated that most zooplankters and many benthic animals were killed by exposure to the 0.5- to 0.6-mg/L concentration normally used to eradicate fish in water reclamation projects. Chironomid larvae were killed by this concentration after 23 h of exposure; 1.0 mg/L killed some species of trichopteran larvae in 5 h. Bottom-dwelling organisms and periphytic cladocerans were more resistant to rotenone, and higher concentrations and longer exposures were required to produce mortalities equivalent to those in other aquatic forms. Schnick (1974) reviewed the piscicidal uses of rotenone and included invertebrate toxicity data.

Although much information exists relating to the effects of rotenone exposure on aquatic invertebrates, many of the data used to support the original registration must be updated to conform to present labeling requirements (Lennon 1967). Marking (1975) proposed a guide or protocol for this type of registration research. The present study is part of a series of investigations designed to determine the toxicity of Noxfish to aquatic nontarget organisms in support of its continued registration as a piscicidal chemical.

Noxfish, an emulsifiable concentrate containing 5% rotenone, was used for all of the toxicity tests. Stock solutions were prepared in acetone daily as needed. The volume of stock needed to yield a particular concentration was added volumetrically to each of the test vessels.

Static tests were performed in a manner similar to that outlined by Lennon and Walker (1964) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Most of the test organisms were collected from the wild, but *Daphnia*, *Catenula*, *Physa*, *Helisoma*, and *Palaemonetes* came from laboratory or pond cultures. All organisms were held under laboratory conditions for periods of 5 to 14 days before being used for exposures. Ten organisms were added to each of a series of 3.7-L (1-gal) glass jars (test vessels) containing 3 L of limed water (20 mg/L total hardness; pH 6.6). Test organisms were acclimated to their new surroundings for 16 h. As the toxicant was added, the test solutions were stirred gently to ensure homogeneity. Two controls, one with acetone and another with untreated water, were included for each of the tests. Temperature of the test solutions was controlled at 16 ± 1°C by immersing the jars in a

chilled water bath. The absolute determination of death for mollusks was difficult. They were considered dead when a gentle probing of the foot or other appendage with a sharp instrument elicited no response. Dead organisms were removed daily and discarded.

The method of Litchfield and Wilcoxon (1949) was used to derive LC50's (the calculated concentration in water that produced 50% mortality of test organisms) and 95% confidence intervals.

Of the invertebrates in our study the most sensitive species was an ostracod (*Cypridopsis* sp.), with a 96-h LC50 of only 0.34 mg/L (Table 1). The other invertebrates were more tolerant of rotenone than most fish. The most resistant organisms exposed were a snail (*Helisoma* sp.) and the Asiatic clam (*Corbicula manilensis*), for which the respective 96-h LC50's were 7.95 and 7.50 mg/L. Marking and Bills (1976), who reported LC50 values for rotenone for more than 20 species of freshwater fish, showed that common carp (*Cyprinus carpio*) was one of the most sensitive, with a 96-h LC50 of only 0.05 mg/L; the black bullhead (*Ictalurus melas*) was much more resistant (LC50 = 0.39 mg/L). Values for channel catfish (*I. punctatus*), bluegill (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*) were intermediate (0.164, 0.141, and 0.142 mg/L, respectively).

The values for the Asiatic clam and the helisomid snail were more than 150 times that of the carp and about 50 times that of the other three fish species. The remaining invertebrates were 2 to 20 times as tolerant as fish. Larvae of the southern leopard frog (*Rana sphenocephala*) were 3 to 10 times more tolerant than the fish used by Marking and Bills (1976).

Table 1. Acute toxicity of Noxfish to aquatic invertebrates and frog larvae in limed water in static tests at 16 ± 1° C.

Organism	LC50 and 95% confidence interval (mg/L) at				
	1 h	3 h	6 h	24 h	96 h
Flatworm *Catenula* sp.	— —	8.95 8.27–9.68	6.40 4.72–8.68	5.10 3.70–7.03	1.72 1.15–2.57
Daphnid *Daphnia pulex*	0.118 0.102–0.137	0.0960 0.0807–0.114	0.0360 0.0317–0.0409	0.0275 0.0239–0.0316	— —
Ostracod *Cypridopsis* sp.	2.80 2.35–3.34	2.55 2.11–3.08	2.15 1.80–2.56	0.490 0.299–0.803	0.340 0.280–0.557
Freshwater prawn *Palaemonetes kadiakensis*	28.3 22.8–35.0	24.0 19.9–28.9	6.35 5.43–7.43	5.15 4.44–6.00	1.12 0.760–1.65
Dragonfly naiad *Macromia* sp.	— —	275 230–329	34.0 19.6–58.9	4.70 1.45–15.2	1.00 0.730–1.59
Backswimmer *Notonecta* sp.	105 86.5–128	21.0 17.7–25.0	9.00 6.79–11.9	3.42 2.27–5.15	1.58 0.727–3.44
Caddisfly larva *Hydropsyche* sp.	10.7 7.98–14.5	8.00 6.69–9.56	3.55 2.88–4.38	— —	0.605 0.329–1.17
Whirligig beetle, adult *Gyrinus* sp.	47.5 32.6–69.2	8.30 5.42–12.7	8.00 5.51–11.6	3.55 2.05–6.15	0.700 0.400–1.21
Snail *Physa pomilia*	— —	— —	— —	6.35 5.61–7.19	4.00 3.45–4.63
Snail *Oxytrema catenaria*	— —	— —	— —	— —	1.75 1.00–3.06
Snail *Helisoma* sp.	— —	33.5 28.0–40.1	33.5 28.0–40.1	30.0 24.1–37.3	7.95 4.63–13.7
Buckley's filter clam *Elliptio buckleyi*	— —	— —	— —	— —	2.95 2.23–3.90
Flattened filter clam *Elliptio complanata*	— —	— —	— —	— —	2.00 1.53–2.61
Asiatic clam *Corbicula manilensis*	— —	— —	— —	— —	7.50 5.74–9.81
Southern leopard frog larva *Rana sphenocephala*	0.830 0.795–0.867	0.775 0.740–0.812	0.635 0.596–0.677	0.580 0.494–0.680	0.500 0.423–0.591

On the basis of these laboratory toxicity tests we conclude that most invertebrates should be safe during applications of rotenone. We suspect that the benthic forms are even more resistant when they reside in a natural substrate. However, fish and invertebrates are more sensitive to chemicals in laboratory tests than in the natural environment, and the ultimate safety should be evaluated under field conditions.

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Accepted 12 November 1981

Simple, Versatile Microscope Stage for the Identification of Pinned Adult Insects

Species determination of pinned adult aquatic insects often requires detailed examinations at high magnification. Many of the key morphological characters (such as palpal, antennal, or tarsal segments) are found on the ventral side and are often difficult to view. Specimens must be examined at various angles or oriented at peculiar angles to the source of illumination to provide suitable contrast for examination of these minute parts.

Several commercial models of insect microscope stages, based on the types depicted by Oldroyd (1958), are available for use in holding and orienting specimens on the stage plate. The simpler models often consist of corks, lumps of clay, or plasticine on small, angled supports. The more expensive models, which sometimes have cork mounting stages, may incorporate complex pivoting beams ("universal holders") or ball-and-socket components to provide flexibility. The simpler models necessitate frequent repinning and reorientation, which often result in specimen breakage. Although the pivoting beam mechanism is the most versatile of conventional holders, the beams and their supports often interrupt the light path and make it difficult to manipulate the specimen. Specimens mounted on stages are often elevated near the upper limit of the "working range" of the microscope, and some structures may be outside the focusing range of the microscope. The eye pieces may have to be raised

above normal height, and this change may cause discomfort to the viewer.

Because available models did not prove completely satisfactory, I developed a modification to meet my needs. The device consists of three slide rings and a clay holder and provides versatility and ease for handling and viewing specimens. It is inexpensive and easy to build.

Mahogany (0.63 cm thick) was used to construct the modified stage. The diameter of the stage ring (Fig. 1) was based on the size of the aperture that held the original stage plate that it replaces. The diameter of the hole in this ring was based on the amount of hoizontal movement of the device desired (slightly in excess of the field diameter at low power). Diameters of the holes in the upper and lower slide rings were based on the diameter of the bottle cap that was used to connect them. Outside diameters were adjusted so that extreme movement in any direction would not uncover the hole in the stage ring. After the three rings were sawed out and sanded, the holes in the two slide rings were enlarged enough to produce a press fit between them and the bottle cap. Floor wax was rubbed into the surfaces to protect them from soiling and to reduce friction. Wax should not be applied to the holes in the slide rings. A small amount of a quick setting epoxy adhesive was used to coat the interior of the holes in the two slide rings. The bottle cap was then thrust through the upper slide plate, through the hole in the stage ring, and then into the hole in the bottom slide ring. The distance separating the three rings was adjusted to allow free, but not excessive, sliding because movement is necessary for proper orientation of specimens. After the adhesive had set, the bottle cap was filled with modeling clay. The stage ring was then placed in the hole that held the original stage plate.

This microscope stage incorporates many of the advantages of the older devices. I have used it successfully for a number of years on several brands of binocular microscopes. Pinned specimens can be tilted to convenient viewing angles, rotated through 360° (horizontal plane), or moved off center about 7 mm in the same plane. Specimens can easily be positioned within the focal plane of the microscope because the point or head of the insect pin can be thrust into the clay about 16 mm.

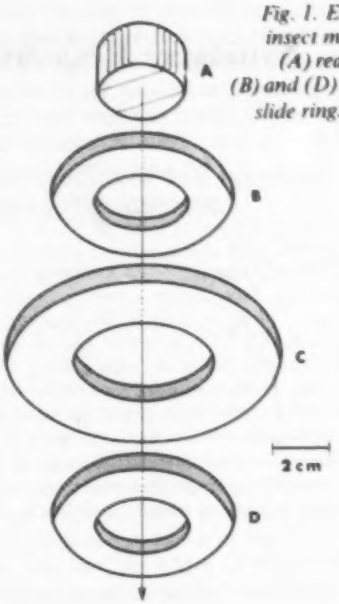

Fig. 1. Exploded view of insect microscope stage: (A) reagent bottle cap; (B) and (D) top and bottom slide rings; (C) stage ring.

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Oldroyd, H. 1958. Collecting, preserving and studying insects. The Macmillan Co., New York. 327 pp.

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Accepted 12 November 1981

Avoidance Response of Rainbow Trout to Phenol

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ABSTRACT: An eight-concentration intermittent-flow proportional diluter was modified to provide continuous flow to four separate avoidance chambers and used to test the avoidance response to phenol of rainbow trout (*Salmo gairdneri*) in three separate 5-day tests. When single fish had been acclimated for 24 h, a flow of phenol was initiated on one side of each avoidance chamber and well water was started on the other. After 48 h the phenol and well water sides were switched, and the experiment was continued for another 48 h; the positions of the fish in the chambers were monitored photographically throughout the test. Results of tests with 12 rainbow trout indicated that the threshold avoidance level was between 6.5 and 3.2 mg/L phenol, which was between 58 and 73% of the phenol 96-h LC50 for rainbow trout. Since long-term effects for rainbow trout exposed to phenol have been documented at concentrations as low as 0.2 mg/L, the avoidance tests were not nearly as sensitive as toxicity tests with embryos and larvae.

Fish may actively avoid effluents that contain toxic chemicals or are low in dissolved oxygen, extreme in temperature or pH, or exhibit other water quality characteristics such as excessive dissolved carbon dioxide or nitrogen. The avoidance of contaminated areas may force fish into habitats that are not favorable for survival, thus eliminating some previously suitable fish habitat (Sprague et al. 1965). On the other hand, Sprague and Drury (1969) demonstrated that fish are attracted to concentrations of some chemicals that are lethal. Since there does not appear to be any a priori way of determining whether fish will avoid or be attracted to harmful discharges, tests of avoidance reactions of representative fish species may be important indicators of potential effects of aquatic pollutants.

Avoidance studies employ either shallow or steep gradients, both of which mimic different types of natural situations (Larrick et al. 1978). Fish have been tested in groups (Ishio 1964; Rice 1973), but most investigators have elected to test fish individually (Kleerekoper et al. 1972; Larrick et al. 1978; Westlake and Lubinski 1976). With one exception (Cripe 1979), most investigators have chosen to run avoidance trials for less than 1 h (Folmar 1976; Hansen et al. 1972; Larrick et al. 1978; Rice 1973; Sprague and Drury 1969; Westlake and Lubinski 1976). Short-term exposure tests provide information that applies primarily to intermittent discharges (such as cooling waters from power plants and accidental spills), whereas longer term avoidance tests are more applicable to continuous discharges (such as municipal sewage effluents, oil refineries, and some chemical and manufacturing processes). To test the long-term avoidance response of fish to toxicants I modified an intermittent-flow proportional diluter (Mount and Brungs 1967) to provide continuous flow and to test the avoidance response of fish to four different toxicant concentrations simultaneously. Rainbow trout (*Salmo gairdneri*) were exposed to four sublethal concentrations of phenol in three separate 5-day tests to determine their avoidance threshold for phenol.

Materials and Methods

A diluter similar to that described by Mount and Brungs (1967) was modified to deliver four concentrations of toxicant plus four streams of well water to four avoidance chambers, each chamber receiving well water on one side and continuous toxicant flow on the other (Fig. 1). The mixing cell for the highest concentration of toxicant (the left-most mixing box in Fig. 1) was supplied with toxicant directly from the toxicant head tank; the three mixing cells for lower concentrations of toxicant (75, 50, and 25% of the highest concentration) were supplied by the diluter. The calibration was adjustable, depending on individual test requirements. The delivery cells for well water were all supplied by a submersible pump in a well water reservoir. The characteristics of the well water are presented in Table 1. A toxicant stock solution was prepared by diluting phenol with well water and pumping that solution to the toxicant head tank with a positive displacement piston pump. The pumping rate was dependent on water flow to the head tank and on the test concentrations desired.

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Distribution Apparatus

Each mixing cell was equipped with three glass delivery tubes (5 mm ID) and an overflow tube (10 mm ID) mounted in neoprene stoppers. The height of the three delivery tubes in each mixing cell was about 10 mm from the bottom of the mixing cell to ensure continuous flow, whereas the overflow tube was near the top of the mixing cell. Plastic tubing (7 mm ID) was used to deliver the toxicant or dilution waters to the test chambers, and the tubing was connected to the mixing cells in such a way that the toxicant and well water supplies to the two sides of each avoidance chamber could be switched during the test without disturbing the fish.

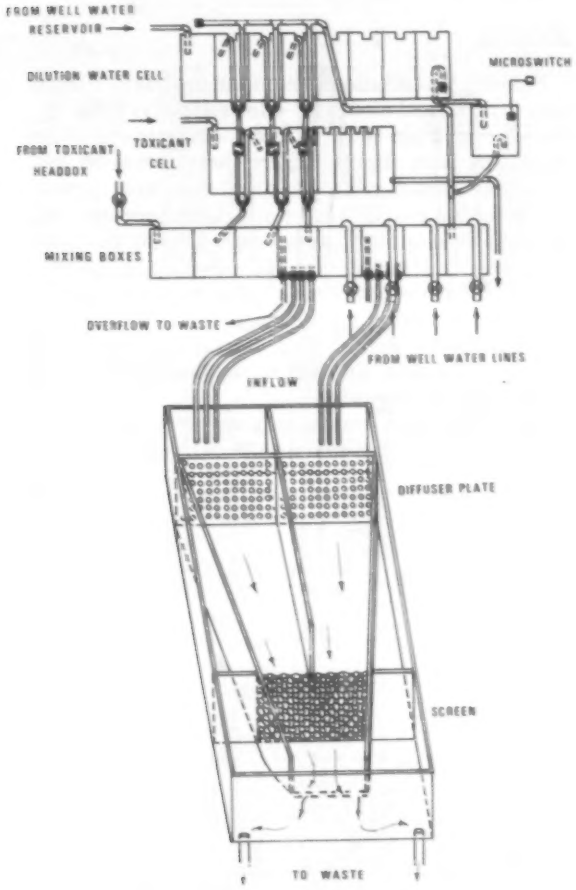

Fig. 1. Schematic diagram of the modified diluter and test chambers used in 5-day continuous-flow avoidance studies.

Avoidance Test Chambers

The avoidance chambers (Fig. 1) were glass tanks 30 x 61 x 30 cm modified in the following ways. The streams of toxicant and well water were separated from each other on the inflow end of the chamber by a glass divider 30 cm long and 10 cm high. Two plastic flow-diffuser plates with about 100 evenly spaced holes (about 1 mm in diameter) were mounted 10 cm from the inflow end of the chamber. Behind these plates the plastic tubes from the toxicant or well water mixing cells were attached to another flow diffuser plate 11 x 13 cm, with 20 2-mm holes (this second diffuser plate is not shown in Fig. 1). Along the length of the chamber, the width of the streams of toxicant and well water was reduced from 15 to 6 cm by glass plates, which increased the water velocity and minimized mixing of the streams of toxicant and well water. At the downstream end of the avoidance chamber a large-mesh stainless steel screen prevented the fish from entering the outflow area. The water depth in the chamber was maintained at 5 cm by an inclined glass spillway that discharged into the waste outflow area. The width of the spillway was 11 cm at the entrance and 6 cm at the exit. A piece of glass 15 x 2.5 cm was mounted 0.5 cm deep between the end of the divider plate and the screen to minimize surface disturbances and mixing. The distance between the divider plate and the screen was 15 cm, which allowed the test fish an access area of 15 x 4.5 cm to either the toxicant or the well water in the avoidance chambers. Opaque plastic sheets inserted between the chambers visually isolated the test fish from each other.

Behavior Monitoring

A Sanyo 8-mm movie camera with a strobe flash attachment and wide-angle lens was mounted 210 cm

Table 1. Characteristics of dilution water used in avoidance experiments.

Chamber	Water quality characteristics[a]						
	Temp (°C)	Dissolved oxygen mg/L	Dissolved oxygen % sat.	Free CO_2 (mg/L)	pH	Hardness (mg $CaCO_3$/L)	Conductivity (μmho/cm at 25°C)
Control	12	7.2	94	3.8	8.0	715	1,151
Toxicant	12	6.5	85	3.1	8.1	707	1,212

[a] Values are means for the control chambers and for test chamber A, the highest concentration toxicant chamber.

above the surface of the water in the chambers and equipped with an electronic timer to enable single-frame exposures at intervals from 1 min to 1 h. A battery with a charger was connected to the camera to ensure continuous operation for up to 4 days. The camera was adjusted to expose 1 frame every 10 min, and a curtain was drawn around the chambers to block out any exterior disturbances. A sign indicating the date, toxicant, and species was placed on the surface of the chambers and photographed to identify the test period.

Test Procedure

An acclimation period of 24 h was selected (based on observations of fish movement in the test chamber) to ensure that the fish were completely familiar with the experimental environment. To initiate the acclimation period one well-fed fingerling (7.5–9.3 cm) rainbow trout was placed in each chamber. The trout were reared in well water (Table 1) from eggs obtained through the courtesy of the Wyoming Game and Fish Department. The photoperiod was 16L:8D. During the acclimation period the toxicant head tank contained only well water, so that water conditions on both sides of the avoidance chamber were identical. The toxicant supply pump, which delivered phenol to the toxicant head tank at a predetermined rate, was activated after 24 h. The amount of phenol necessary to bring the head tank concentration immediately to the desired test level was also added. The avoidance chambers reached the desired test concentrations within 10 min, as confirmed by earlier dye studies and chromatographic analyses of phenol. This initial exposure was continued for 48 h, with occasional observations to assure proper operation. After this 48-h initial exposure period the toxicant and dilution water sides in the avoidance chambers were switched by interchanging the plastic tubing on the mixing cells, and the fish were observed for a 48-h final exposure period.

Phenol Determinations

During a pre-test calibration period, numerous samples taken concurrently from mixing cells and avoidance chambers showed that the phenol concentrations were always within 2% of each other. Therefore, during the tests daily samples to check phenol concentrations were obtained by sampling from the appropriate mixing boxes. All samples were analyzed by high-pressure liquid chromatography.

Data Analysis

A microfilm viewer was used to examine the position of each fish in each tank at 10-min intervals during acclimation, initial exposure, and final exposure. After the position of each fish was recorded for each 10-min observation, the number of observations for each fish in the toxicant stream and well water stream was converted to a percentage. A chi-square analysis was used to determine if the percentage of observations in control (well water) and in toxicant during each test period differed significantly from an expected equal distribution on both sides of the avoidance chamber.

Results

Phenol concentrations were measured daily during tests I and III and on days 2–4 during test II (Table 2). During tests I and II the phenol concentrations in the head tank were slightly higher than in test III but remained reasonably stable (standard deviation = 0.31 mg/L in both tests). Consequently, the phenol levels in the mixing boxes did not fluctuate substantially. During test III, however, a greater variation in the head-tank phenol concentration (standard deviation = 1.10 mg/L) was caused by a malfunctioning pump. This fluctuating head-tank concentration was reflected in the measured phenol levels in the mixing cells.

Three separate avoidance tests were performed (Fig. 2). Of the 12 rainbow trout exposed in these 3

Table 2. Phenol concentrations (mg/L) in head tank and mixing cells supplying avoidance chambers.

Test and day	Head Tank	Mixing cell[a]			
		A	B	C	D
Test I					
1	7.9	7.9	6.3	4.3	2.3
2	8.5	8.1	6.4	4.4	2.2
3	8.5	8.4	6.3	4.4	2.2
4	8.5	8.2	6.8	3.9	2.1
Mean	8.4	8.1	6.4	4.2	2.2
SD	0.31	0.23	0.24	0.24	0.06
Test II					
2	8.6	8.3	6.5	4.3	2.3
3	8.3	8.4	6.6	4.2	2.4
4	8.0	7.5	6.4	3.5	2.2
Mean	8.3	8.1	6.5	4.0	2.3
SD	0.31	0.50	0.12	0.45	0.08
Test III					
1	7.3	6.5	4.9	3.1	2.1
2	5.7	5.4	4.4	2.9	1.6
3	8.4	8.5	6.6	3.8	2.3
4	7.1	6.6	5.2	3.1	1.6
Mean	7.1	6.8	5.3	3.2	1.9
SD	1.10	1.27	0.92	0.41	0.33

[a] Nominal concentrations for avoidance chambers in all tests were A = 8 mg/L; B = 6 mg/L; C = 4 mg/L; D = 2 mg/L.

tests, 9 showed a significant side preference during the 24-h acclimation period. However, all fish spent some time on each side of the tank.

The three fish exposed to the highest phenol concentrations during the initial exposure periods of the three tests (Chamber A in Table 2: 6.8–8.1 mg/L) showed strong avoidance behavior: 70–93% of the observations were made in control well water. The avoidance response was somewhat reduced in each instance after the toxicant and well water sides were switched during the final exposure period, but all fish changed their position in the tank to avoid the phenol. This position change during the final exposure period required that the fish overcome any location preference that had developed during the preceding 48 h.

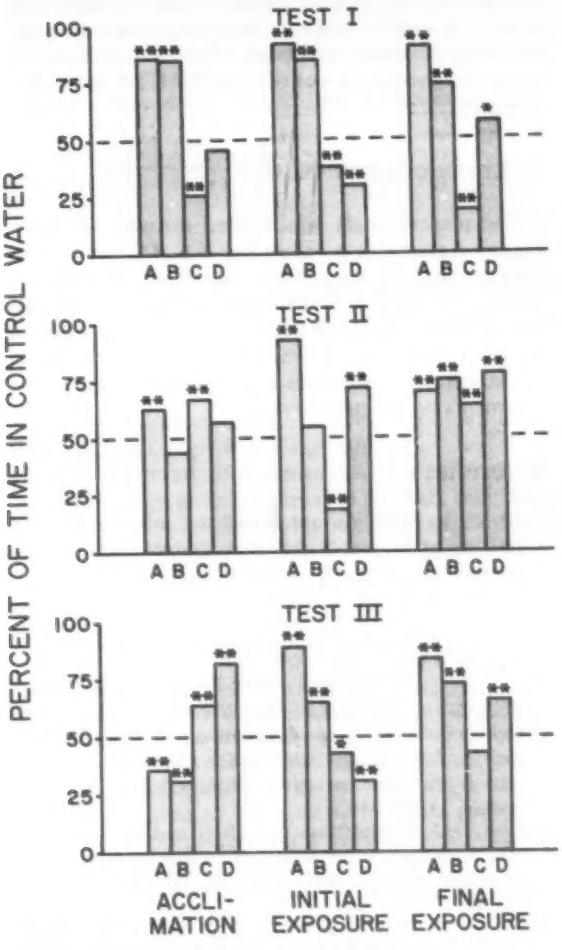

*Fig. 2. Percent of observations of fish in control well water (avoiding phenol) during three avoidance tests. Test periods: Acclimation, 24-h with control water on both sides of each chamber; initial exposure, 48-h with phenol delivered to one side of each chamber; final exposure, 48-h with phenol and control water sides switched from those of the initial exposure. Nominal phenol concentrations in all tests: A = 8 mg/L; B= 6 mg/L; C= 6 mg/L; D= 2 mg/L (see Table 2 for measured concentrations). Number of observations exceeded 120 for each mean value. Significant differences from 50% value: * = P ≤ 0.05; ** = P ≤ 0.01.*

With one exception (test II, initial exposure) the rainbow trout exposed to 5.3–6.5 mg/L phenol (chamber B) also showed significant phenol avoidance, although the data suggest a weaker avoidance response than was observed in the tanks with higher concentrations of phenol (Fig. 2). Again, the trout switched sides during the final exposure period to avoid the phenol.

The fish responded ambivalently to 3.2–4.2 mg/L phenol (chamber C). During all of test I and during the initial exposure periods of tests II and III the trout apparently preferred the phenol to the well water and the results were statistically significant. During tests II and III at 4.0–4.2 mg/L phenol the fish usually stayed on the same side of the tank after the phenol side was switched for the final exposure period, thus now showing a significant preference for well water.

The trout exposed to 1.9–2.3 mg/L phenol (chamber D) showed similar reactions; the phenol side was preferred during the initial exposure period of tests I and III and fish did not relocate after phenol sides were switched. On the other hand, the fish in test II significantly avoided the 2.3-mg/L phenol side during both the initial and final exposure periods. Overall, however, the results obtained at the two lowest exposure levels suggest a possible location preference rather than avoidance of, or attraction to, phenol.

For all tests and all phenol test concentrations, the frequency of entry by the trout into the phenol side of the tank was evenly distributed throughout each 48-h test period. Upon entry into the phenol, particularly the two highest concentrations (chambers A and B), the trout showed obvious signs of detecting the phenol such as coughing, rapid breathing, erratic swimming, jumping out of the water, loss of orientation, and loss of equilibrium. These behavioral responses were more apparent when the phenol was initially introduced.

Discussion

The present avoidance experiments with phenol indicate that the threshold avoidance level for rainbow trout lies somewhere between concentration ranges of 5.3–6.5 and 3.2–4.2 mg/L. In an earlier study using the same dilution water, DeGraeve et al. (1980) reported the 96-h flow-through LC50 for juvenile rainbow trout to be 8.9 mg/L phenol. In the same study we also observed reduced growth in newly hatched rainbow trout exposed for 60 days to 0.20 mg/L phenol (2.2% of the LC50). Since the

phenol range of 5.3 to 6.5 mg/L was the lowest that produced a significant avoidance response, the threshold avoidance level was somewhere between 59 and 73% of the 96-h LC50 value for rainbow trout established by DeGraeve at el. (1980). Thus, the avoidance tests were clearly not as sensitive as toxicity tests with embryos and larvae for predicting threshold effects.

Hasler and Wisby (1949) trained the bluntnose minnow (*Pimephales notatus*) to discriminate between phenol and p-chlorophenol at concentrations as low as 0.0005 mg/L when phenol was associated with food and p-chlorophenol with punishment. However, the ability to detect phenol at this extremely low level does not imply that fish will avoid sublethal or even lethal phenol concentrations. Jones (1951) reported that the minnow (*Phoxinus phoxinus*) failed to avoid from 4 to 400 mg/L phenol in an avoidance tube. Using the same test species, Stott and Buckley (1979) found that although 10 mg/L phenol killed some fish, concentrations of 4, 8, and 10 mg/L did not elicit an avoidance response. Similarly, exposure to 0.001–10.0 mg/L phenol failed to induce significant avoidance reactions in rainbow trout (Sprague and Drury 1969).

The lower avoidance threshold level observed in my study could be related to the test duration. Sprague and Drury (1969) tested their fish for only 10 min, Jones (1951) exposed his for 4 h, and Stott and Buckley (1979) conducted their tests for a maximum of 12.5 h. The longer exposure times used in the present study (96 h) may make avoidance tests more sensitive, possibly because the fish became more familiar with their environment. If that is the case, then longer avoidance tests may be more representative of actual environmental circumstances.

The results of the present laboratory study are basically in agreement with most published studies of avoidance reactions of fish exposed to phenol. Only a few field observations have been reported on the effects of phenol on fish, and these reports are conflicting. Kalabina (1935) concluded from his observations that fish were avoiding 0.2 mg/L phenol in a river. But Kromback and Barthel (1964), investigating an accidental phenol spill, found that, with the exception of salmonids, there was essentially no harm to the fauna in 3–10 mg/L phenol, and that where phenol levels ranged from 0 to 3 mg/L there was "practically no damage" to the fauna, including salmonids. My data tend to support the latter findings, since fingerling rainbow trout avoided acutely toxic phenol concentrations, but remained in phenol solutions which would have had harmful effects on fry survival and growth. In applying my results to field situations, it is important to recognize that fish motivated by hunger or by reproductive or territorial defense instincts may not avoid the phenol concentrations that elicited avoidance in the present study. Thus, fish might live for extended periods in phenol concentrations approaching the LC50 for that species. However, reproduction and growth would probably be affected under those circumstances (DeGraeve et al. 1980).

The duration of my experiments (4 days) and the 6-day sampling period of Kromback and Barthel (1964) may have been too brief to adequately assess the potential for long-term avoidance reactions under continuous exposure conditions. Fish in longer term avoidance studies, however, might demonstrate acclimation to pollutants that could effectively increase the threshold avoidance concentrations (DeGraeve and Ward 1977).

Acknowledgments

The present study was funded through an Interagency Agreement between the U.S. Department of Energy and the U.S. Environmental Protection Agency under Contract DE-AS20-79-LC-01761 to the University of Wyoming. I thank Joe Meyer for performing the phenol analyses, Mark Boyce for consultation on the statistical procedures and Harold Bergman for comments on the manuscript.

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Accepted 1 October 1981

Comparison of Cleithra and Scales for Age and Growth Analysis of Yellow Perch[1]

Aging fish in temperate regions through the identification of annual growth rings on scales was first described in the late 19th century (Ricker 1975). Other calcified tissues have been used to age fish: opercula, fin rays, vertebrae, otoliths, and cleithra (Bagenal and Tesch 1978). Scales are commonly used because they can easily be collected without sacrificing fish. It is sometimes difficult to identify annual growth rings on scales, and other calcified tissues may provide better means of aging. LeCren (1947) determined that opercula provided a good general method for ascertaining the age and growth of European perch (*Perca fluviatilis*). The use of cleithra and scales for assessing the age and growth of muskellunge (*Esox masquinogy*) resulted in equivalent data for younger fish; however, cleithra appeared to be superior to scales for aging older fish, whose annuli were more difficult to identify (Harrison and Hadley 1979).

The age of yellow perch (*Perca flavescens*) is usually determined from their scales; however, scales from older fish can be difficult to age. Because we believed that the use of cleithra might be an alternate aging method that could improve accuracy, we compared this method to the use of scales for both aging and backcalculating growth of yellow perch.

Perch were collected by gill net from West Lake Okoboji, Iowa, in 1979 and 1980. Cleithra were excised according to Casselman's (1974) method for esocids. Forceps were used to tear away dermal tissue and muscle. Excess tissue was scraped from cleithra under running water. The cleithra were air dried and stored in envelopes coded for later identification. Scales were removed according to the method described by Lagler (1956) and placed in coded envelopes apart from the cleithra. Total body length (in millimeters) was recorded.

Cleithra were examined for annuli under a 10–40x dissecting microscope with light transmitted upward from below the stage. The outer limit of each translucent band was considered as the point of annulus formation (Fig. 1). The origin was identified as a V-shaped notch in the bone. Distances from the origin to the annuli and to the anterior tip from the origin were measured with a dial caliper (accuracy, ± 0.05 mm). Scales were placed between glass microscope slides and examined at a magnification of 48x with a microprojector. Paper strips laid across the projected image were marked at the locations of the origin, annuli, and anterior scale margin, and the distances were measured on the strips to the nearest millimeter.

Both investigators independently determined the age of 100 fish by using the cleithra and scales separately; discrepancies in aging were resolved by using both cleithra and scales. After the first reading, independent age determinations by both investigators agreed for 87% of the cleithra and 86% of the scales, indicating that the investigators' ability to age perch with the two structures was similar. The age of fish determined from both cleithra and scales agreed in 80% of the paired samples at the first reading,

[1] Journal Paper No. 10374 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project No. 2345. The work was financed by the Iowa State Conservation Commission.

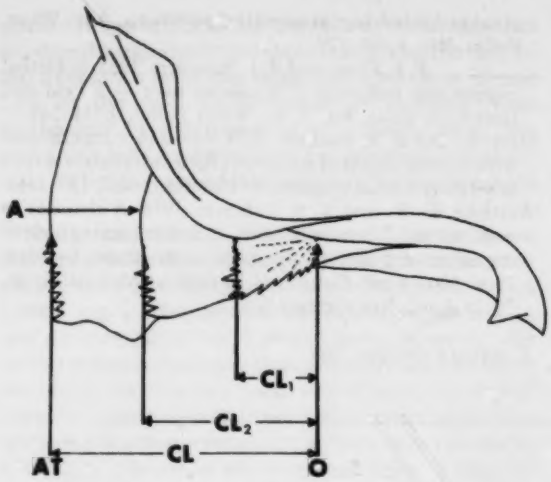

Fig. 1. Ventral view of the yellow perch cleithrum used in backcalculation of body depth. A = annulus, AT = anterior tip, O = origin, CL = total length of cleithrum, CL_1 and CL_2 = annulus measurements for years I and II.

indicating a high degree of similarity between the two structures.

The major value in the use of cleithra as an aging technique for yellow perch is as a means of verifying scale readings and of improving confidence in age determinations, especially for older fish. For perch of ages IV and V, cleithra probably are superior to scales in the identification of the later annuli, although the first annulus becomes increasingly difficult to identify as age advances and the thickness of the bone increases. Disadvantages associated with the use of cleithra are the need to sacrifice the fish and the greater time required to collect and clean the bones. The potential for increasing confidence in aging by using both scales and cleithra may justify the sacrifice of fish and greater expenditure of time in studies where the best possible estimates of age and growth are needed.

Table 1. Backcalculated mean total lengths at annulus formation based on measurements of cleithra and scales of yellow perch.

Age	No. of Fish	Total length (mm)	
		Cleithra	Scales
I	140	63.2	58.3[a]
II	140	149.8	138.0[a]
III	65	196.1	191.6
IV	32	215.2	216.9
V	4	237.0	233.3

[a] Estimated total lengths less than those on cleithra ($P \leq 0.0001$). Other values developed by the two methods were not significantly different (Student's *t*-test).

Backcalculations of annual lengths were computed by direct proportion or $L_i = (s_i/S)L$, where L_i = total length of fish at annulus i, s_i = distance from the origin to annulus i, S = distance from the origin to the anterior margin, and L = total length of fish at capture. Computed mean lengths at annulus formation at ages I and II were significantly greater when cleithra were used than when scales were used (Table 1). Evaluation of the measurements between the origin and anterior tip of cleithra indicated that the difference in back-calculated growth between cleithra and scales probably was due to an inaccurate identification of the origin of the cleithrum. The inaccuracy resulted in an over-estimate of total cleithrum length, as well as the distance to each annulus, and overestimates of total length at each annulus. The actual origin of the cleithrum probably is a point anterior to the V-shaped notch used in the present study.

Acknowledgments

We thank the Iowa State Conservation Commission and Iowa Lakeside Laboratory for their support and Kenneth Carlander, John Nickum, and James Mayhew for their comments on the manuscript.

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Accepted 16 December 1981

[2] The Unit is jointly sponsored by Iowa State University, the Iowa State Conservation Commission, and the U. S. Fish and Wildlife Service.

Aeration of Water Supplies for Fish Culture in Flowing Water

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ABSTRACT: An analytical approach to the reaeration of flowing water for aquaculture is presented, together with a rational method for the assignment of dissolved oxygen minima on the basis of respiratory characteristics of fish. Methods for calculation of expected oxygen transfer capabilities of gravity devices and mechanical units are given.

The water requirements for intensive fish culture with respect to the oxygen consumption of fish have been widely documented (Haskell 1955; Willoughby 1968; Piper 1970; Westers 1970; Liao 1971; Westers and Pratt 1977) and the managers of modern hatcheries generally have a working knowledge of this information. The extent to which water can be reused before intolerable levels of un-ionized ammonia accumulate is similarly well understood (Westers and Pratt 1977). Quantitative knowledge concerning the transfer of atmospheric oxygen to water in which the dissolved oxygen (DO) level has been reduced below the equilibrium concentration is required for successful fish culture. In this paper an analytical approach to the aeration of flowing water is offered.

In contrast to the conditions for intensive husbandry of terrestrial animals, in fish farming the supply of the oxygen carrier, water, is limited. Therefore, the first consideration in evaluating a water supply for aquaculture is its volume (Haskell 1955; Willoughby 1968; Piper 1970; Westers 1970; Liao 1971; Westers and Pratt 1977). The fish production that a given volume of water can support depends on the amount of DO in the water, the oxygen consumption rate of the fish, and the efficiency with which fish can extract DO from the water.

Solubility of Oxygen in Water

Although oxygen occupies nearly 21% of the atmosphere by volume, it is only sparingly soluble in water. As with all dissolved gases, its solubility in water decreases as the temperature increases, as atmospheric pressure decreases, or both. Cold water can therefore hold more DO than warm water, and at sea level water can hold more DO than at higher elevations (Table 1). Most fish culturists use similar tables with correction factors for different elevations. I believe a more precise method for determining the solubility of oxygen in water of any temperature at any altitude would be by calculation. Several authors provide empirical formulae for the determination of the equilibrium concentration of oxygen in water (Truesdale et al. 1955; Whipple et al. 1969; Liao 1971) but the expression given by Truesdale et al. (1955) gives the most widely accepted values:

$$Ce = 14.161 - 0.3943T + 0.007714T^2 - 0.0000646T^3$$

where Ce = equilibrium concentration of oxygen (milligrams per liter) at a pressure of 760 mm of mercury, and T = water temperature in degrees Celsius.

Table 1. Equilibrium concentrations (Ce) of dissolved oxygen in water at various temperatures and three elevations (after Truesdale et al. 1955; Liao 1971).

Temperature (°C)	Ce (mg/L) of DO at elevations[a] of		
	0	500	1000
0	14.16	13.29	12.51
2	13.40	12.58	11.84
4	12.70	11.92	11.23
6	12.05	11.31	10.66
8	11.47	10.76	10.13
10	10.92	10.25	9.65
12	10.43	9.78	9.22
14	9.98	9.36	8.82
16	9.56	8.97	8.45
18	9.19	8.62	8.12
20	8.84	8.30	7.82
22	8.53	8.00	7.54
24	8.25	7.74	7.29
26	7.99	7.50	7.06
28	7.75	7.27	6.85
30	7.53	7.07	6.65

[a] Meters above sea level.

The most accurate way to correct for pressure is to measure the barometric pressure when the oxygen solubility is to be calculated. The pressure correction factor then is P/760, where P = measured barometric pressure in millimeters of mercury. If a barometer is not available, elevation can be used as an approximation of barometric pressure. Liao (1971) provides the following pressure correction factor: 760/(760 + E/32.8), where E = elevation in feet above sea level (since this is an empirical formula, the original English units are retained).

Water containing DO at the equilibrium concentration is saturated with oxygen. The amount of DO in water is almost universally expressed in concentration units (milligrams per liter), but for fish respiration problems it is more usefully expressed as pressure in millimeters of mercury, which is equivalent to expressing the amount of DO as percent of saturation. Since standard atmospheric pressure is 760 mm Hg and the atmosphere contains 20.946% oxygen by volume, the tension of oxygen in air at standard conditions is 159.2 mm Hg, in accordance with Dalton's Law. The oxygen tension in air at any barometric pressure is that pressure (in millimeters of mercury) multiplied by the decimal fraction 0.20946. To calculate the oxygen tension in water, the percent of saturation (measured DO concentration divided by the calculated equilibrium concentration) is multiplied by the tension of oxygen in air at the site. If a barometer is not available the average barometric pressure can adequately be estimated if the elevation is known. Thus, P/760 = 760/(760 + E/32.8), where P = average atmospheric pressure in millimeters of mercury and E = elevation in feet above sea level.

Oxygen Requirements of Fish

In respiration fish blood picks up oxygen and releases carbon dioxide at the gills and picks up carbon dioxide and releases oxygen at the tissues. The efficiency at which the blood combines with oxygen and carbon dioxide at different tensions therefore determines the reaction of the fish to reduced DO concentrations in the water. Brungs (1971), in a carefully controlled long-term study, found that the growth of fathead minnows (*Pimephales promelas*) was reduced at all DO concentrations below saturation. Andrews et al. (1973) reported that channel catfish (*Ictalurus punctatus*) ate less and grew more slowly at 60% saturation than at 100% saturation. The DO tension in fish culture systems cannot be kept at the saturation level because fish respiration continuously depletes the oxygen present in the water. Minimum oxygen tensions acceptable for adequate growth and health of the fish should be defined as aquaculture facility design criteria.

Fish in warm water can tolerate lower concentrations of DO than can fish in cold water because the tension of a given concentration of oxygen becomes greater as the solubility decreases. This relation has been verified by field observations from the literature. Smith and Piper (1975), Piper (1970), Willoughby (1968), and Westers and Pratt (1977) suggested that aquaculture facilities for trout be designed so that the fish are exposed to a minimum DO concentration of 5.0 mg/L. Buss and Miller (1971) called for aeration at trout hatcheries when the DO concentration was predicted to fall below 5.0–7.0 mg/L. Burrows and Combs (1968) reported that salmon growth was reduced when the DO concentration fell below 6.0 mg/L. Warmwater fish are reported to be able to survive prolonged exposures as low as 1.0 mg/L, but in the range of 1.0–5.0 mg/L growth is reduced (H. S. Swingle, Auburn University, unpublished data). Carlson et al. (1980) found that at 25°C the growth of channel catfish was reduced at a constant DO exposure of 3.5 mg/L but not at 5.1 mg/L.

Piper (1970) reported a water temperature of 10°C and an elevation of 1,500 m above sea level for his station. At this site a minimum DO of 5.0 mg/L is probably a reasonable design criterion for trout culture. A rational basis for assigning DO minima to warmwater aquaculture systems could thus be the degree of oxygen tension in the water supply. For example, the average oxygen tension of water at 10°C, 1,500 m above sea level and a DO concentration of 5.0 mg/L is 72.8 mm Hg, corresponding to a concentration of 3.44 mg/L at a water temperature of 30°C at standard pressure. This analysis compares with that of Downey and Klontz (1981), who recommended a minimum DO tension of 90 mm Hg.

When fish respiration has reduced the DO tension to about 70–90 mm Hg, water can no longer be used for fish culture because at this pressure the fish cannot efficiently extract the oxygen present in the water. The water must be reconditioned by aeration (i.e., more oxygen must be dissolved into the water) if it is to be of further use for fish production.

The extent to which water can be reconditioned with aeration as the only treatment measure depends on the accumulation of un-ionized ammonia. The number of water uses permissible before un-ionized ammonia reaches unacceptably high levels depends on water temperature, feeding rate, protein content of the diet, and especially on the pH of the water.

Aeration Technology

Gravity Devices

Transfer of oxygen into water is a three-stage process in which gaseous oxygen is transferred to the surface film, diffuses through the surface film, and

finally moves into the liquid bulk by convection (Wheaton 1977). The rate of oxygen transfer depends on the surface area over which transfer occurs. Oxygen transfer in aquaculture systems where water is quiescent or moving in laminar flow is too slow to be an important source of DO for fish respiration unless the area of air-water interface is significantly increased by artificial turbulence or agitation. Aeration of water streams used for aquaculture can be accomplished by gravity where the energy released when water loses altitude is used to increase the area of air-water interface or by mechanical devices that spray water into the air or pump air into the water (Wheaton 1977). The most logical means of improving oxygen regimes of cultured fish is by gravity fall of water between production units, which can be provided by the topography at the facility. The extent to which water is reaerated by gravity is a fundamental concern for practical fish culture in flowing water.

Haskell et al. (1960) compared aeration by water passage over a simple weir (Fig. 1A) with that obtained by flow over a splashboard (Fig. 1B) that broke up the water fall part way down, and that from flows over various screens and slat arrangements at the dam. Chesness and Stephens (1971) evaluated several devices for increasing oxygen transfer over a gravity fall, including a splashboard, an inclined sheet of corrugated roofing material (Fig. 1C), a similar corrugated sheet pierced with holes (Fig. 1D), and an open stairstep device referred to as a lattice (Fig. 1E). Tebbutt (1972) studied aeration down closed stairstep arrangements of various heights that he called cascades (Fig. 1F).

The following equation (Downing and Truesdale 1955) can be used to evaluate and compare gravity aeration devices:

$$E = 100 \times \frac{\text{actual increase in DO}}{\text{possible increase in DO}}$$

or $E = 100 \times (Cb-Ca)/(Ce-Ca)$, where E = efficiency, Cb = DO below the device, Ca = DO above the device, and Ce = equilibrium concentration (all in milligrams per liter). Selected data on measured efficiencies of some gravity aerators over various distances of water fall are presented in Table 2.

Practical application of this information requires rearrangement of the efficiency equation to solve for the expected DO concentration below an aeration device of a known efficiency:

$$Cb = \frac{E\,(Ce - Ca)}{100} + Ca$$

The use of this equation is best illustrated by example. Suppose there is a 30.5-cm drop between two ponds, with a simple weir separating them. If the water temperature is 10° C and the elevation is 183 m above

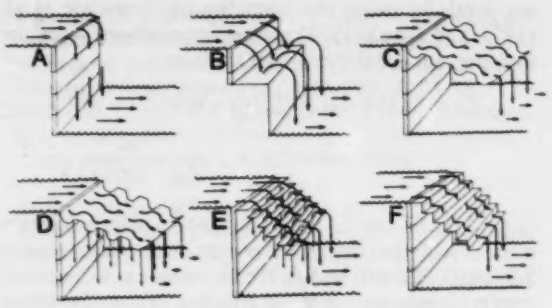

Fig. 1. Diagrams of gravity aerators. A, Simple weir (Haskell et al. 1960; Chesness and Stephens 1971); B, splashboard (Haskell et al. 1960; Chesness and Stephens 1971); C, inclined corrugated sheet (Chesness and Stephens 1971); D, inclined corrugated sheet with holes (Chesness and Stephens 1971); E, lattice aerator (Chesness and Stephens 1971); and F, cascade aerator (Tebbutt 1972).

Table 2. Selected data on measured efficiencies of some gravity aerators over various distances of water fall.

Device and distance of water drop (cm)	Efficiency (%)
Simple weir	
22.9[a]	6.2
30.5[b]	9.3
61.0[b]	12.4
Inclined corrugated sheet[b]	
30.5	25.3
61.0	43.0
Inclined corrugated sheet with holes[b]	
30.5	30.1
61.0	50.1
Splashboard	
22.9[a]	14.1
30.5[b]	24.1
61.0[b]	38.1
Lattice[b]	
30.5	34.0
61.0	56.2
Cascade[c]	
25.0	23.0
50.0	33.4
75.0	41.2
100.0	52.4

[a] Haskell et al. (1960).
[b] Chesness and Stephens (1971).
[c] Tebbutt (1972).

sea level, by using the equation of Truesdale et al. (1955) and Liao's (1971) pressure correction factor, we find that the solubility of DO will be

$$Ce = 14.161 - 0.3943(10) + 0.007714(10)^2 - 0.0000646(10)^3 \times \frac{760}{760 + 600/32.8}$$

or Ce = 10.67 mg/L. If the fish loading in the upstream pond is such that the DO concentration is depressed to 5.0 mg/L, Ca will be 5.0. We know that a 30.5-cm fall over a simple weir is 9.3% efficient (Table 2). Using the equation of Downing and Truesdale (1955), we can determine the DO below the weir (Cb) as follows:

$$Cb = \frac{9.3\,(10.67 - 5.0)}{100} + 5.0$$

or Cb = 5.53 mg/L. However, if a lattice structure (Chesness and Stephens 1971) is constructed between the ponds, the efficiency rating of the 30.5-cm fall will rise to 34%, so

$$Cb = \frac{34\,(10.67 - 5.0)}{100} + 5.0$$

or Cb = 6.93 mg/L. Although the estimated benefit of this gravity aeration device is considerable, most aquaculture sites require mechanical aeration to realize the full production potential of their water supplies.

Mechanical Devices

Mechanical units that agitate the water surface are normally used in flowing water aquaculture systems because of their convenience and ease of installation. Aerators are evaluated and compared on the basis of their ability to transfer oxygen to water. Tests are conducted under standard conditions of 760 mm Hg pressure, 20° C temperature, and zero DO in the water to be aerated. The amount of oxygen added to the water in a given amount of time under a certain power level is measured. The kilograms of oxygen per shaft kilowatt per hour (kg/kW•h) is given by the aerator manufacturer as a measure of its efficiency and can be used to compare units. Actual oxygen transfer depends on the oxygen concentration gradient: as saturation is approached an increasing amount of power is required per unit of DO transferred. Reaeration above 95% of saturation can seldom be justified on a cost basis (Mayo 1979). Westers and Pratt (1977) list 90% of saturation as a reasonable design criterion for reaerated water. Since aquaculture systems operate at the relatively high DO minima of 3–7 mg/L, actual transfer rates will be less than those determined under standard conditions; thus these figures may not be used as design criteria.

Surface aerators are generally rated to transfer 1.9–2.3 kg/kW•h under standard conditions (Eckenfelder 1970). Whipple et al. (1969) found that mechanical aerators in polluted rivers generally provided oxygen transfer rates of 0.61 kg/kW•h or less, but their test water supply was higher in oxygen demand than is usual for aquaculture effluents. Soderberg (1980) reported an average transfer rate of 0.83 kg/kW•h in static-water trout ponds where the fish were heavily fed and aeration began when DO tensions reached 70 mm Hg. Aeration of flowing water should be more efficient because processed water is continually being replaced from upstream rather than being recirculated around the unit, as might occur in static water conditions. To estimate aeration requirements, we may use a conservative value such as 0.6 kg/kW•h or we may estimate oxygen transfer by using the formula presented by Whipple et al. (1969):

$$RT = RS\,\frac{(Ce_t - Ca)\,(1.025^{T-20})\,(0.85)}{Ce_{20}}$$

where RT = actual oxygen transfer, RS = oxygen transfer under standard conditions, Ce_t = equilibrium concentration of DO at aeration site, Ca = DO above aerator, Ce_{20} = equilibrium concentration of DO under standard conditions, and T = water temperature in Celsius degrees.

The following example illustrates the use of this formula. Suppose an aerator is rated by the manufacturer to transfer 2.0 kg O_2/kW•h under standard conditions. The actual oxygen transfer at a site where the water temperature is 10° C, barometric pressure is 725 mm Hg, and the DO in the water to be aerated is 5.0 mg/L, may be estimated as follows:

$$RS = 2.0 \text{ kg/kW•h}$$

$$Ce_t = 14.161 - 0.3943\,(10) + 0.007714\,(10)^2 - 0.0000646\,(10)^3 \times 725/760 = 10.42 \text{ mg/L}$$

$$Ca = 5.0 \text{ mg/L}$$

$$Ce_{20} = 14.161 - 0.3943\,(10) + 0.007714\,(20)^2 - 0.0000646\,(20)^3 = 8.84 \text{ mg/L}$$

$$T = 10°\text{C}$$

$$RT = 2.0\,\frac{(10.42 - 5.0)\,(1.025^{10-20})\,(0.85)}{8.84}$$

and, thus, RT = 0.81 kg/kW•h.

When the actual oxygen transfer rate (RT) has been estimated, an estimate of the aeration capability of a particular unit and set of conditions can readily be obtained. For the above example, suppose that a 1.0-kW unit will be used in a water flow of 4,000 L/min. The DO concentration below the aerator (Cb) is calculated as follows:

$$Cb = Ca + \frac{0.80\ kg}{kW{\cdot}h} \times \frac{10^6\ mg}{kg} \times 1.0\ kW \times \frac{min}{4{,}000\ L} \times \frac{h}{60\ min}$$

$$Cb = 5.0 + 3.38 = 8.38\ mg/L$$

A more practical application of this knowledge might be to size an aerator for a particular job. Suppose that for the same example a unit that will return the DO to 90% of saturation is desired. The amount of oxygen needed would be

$$[(0.9)(10.42) - (5.0)]mg/L \times \frac{kg}{10^6\ mg} \times \frac{4{,}000\ L}{min} \times \frac{60\ min}{h} = \frac{1.05\ kg}{h}$$

The size of the unit required (in kilowatts of shaft power) would be

$$\frac{1.05\ kg}{h} \times \frac{kW{\cdot}h}{0\ 81\ kg} = 1.3\ kW$$

The final step in evaluating an aeration program is to compare the cost of purchasing and operating the equipment with the potential value of the increased fish production anticipated. The relation between shaft power and brake power is given by the motor manufacturer as the efficiency, and this can be used to estimate energy consumption. For example, a 1.30-kW motor that is 75% efficient would draw 1.73 kW of energy. If run continuously on electrical power that was purchased for $0.10/kW•h, the unit would cost $124.80 per month to operate.

Acknowledgment

Preparation of manuscript was aided in part by Hatch Project Alabama No. 497, Auburn University Agricultural Experiment Station.

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Accepted 12 November 1981

Enhancement of Growth of Common Carp by Injection of a Homogenate of Carp Pituitary Glands[1]

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ABSTRACT: The growth rate of small (10-25 g), intact (i.e., not hypophysectomized) common carp (*Cyprinus carpio*) held at 35° C was enhanced by injections of pituitary material obtained from adult carp. Percentage increase over initial length and weight was dose-dependent by 4 weeks after the first injection. The dose-response curve for length increase due to the pituitary material was parallel to the curve demonstrated for injections of bovine growth hormone (bGH). The potency of bGH (determined in a previous study) was from 5.0 to 5.4 times greater per unit weight than the potency of the pituitary material. Condition factor of all fish increased slightly; the greatest increase was apparent among fish receiving the two lowest dosages and the least increase was noted among the control fish.

Numerous studies have demonstrated that the growth of fish is stimulated in response to administration of mammalian growth hormones (GH; for a review, see Donaldson et al. 1979). Fewer studies have been attempted with fish GH. Growth of hypophysectomized male killifish (*Fundulus heteroclitus*) has been promoted by presumptive GH extracted from pituitaries of various marine fish (Pickford 1954; Lewis et al. 1972) and by a pituitary homogenate from perch (*Perca fluviatilis*; Swift and Pickford 1965). Growth rates of hypophysectomized rainbow trout (*Salmo gairdneri*; Komourdjian and Idler 1979) and intact salmon (*Oncorhynchus* spp.) have been increased by administration of various fish pituitary extracts or purified fish GH (Clarke et al. 1977; Higgs et al. 1978).

Powdered common carp pituitary is commercially available and is used extensively to artificially induce spawning in important fish species, but information is not available on the anabolic potency of common carp pituitaries. The present study was undertaken to provide a dose-response relationship for a subsequent study of carp pituitaries and to determine the potency of injections of a homogenate of common carp (*Cyprinus carpio*) pituitary glands for enhancement of growth rate of intact young common carp.

Methods

Pituitary Preparation

Adult carp (1 kg or more) were collected in July, August, and October 1975 and April through October 1976 by electrofishing on the Mississippi River at Monticello, Minnesota, and were held alive in a cage in the river until sacrificed. Pituitary glands were extracted from 10 min to 5 h after capture and immediately frozen on dry ice.

The wet pituitaries were stored at −80° C until November 1976, when they were lyophilized and stored again at −20° C. In April 1977 (2 weeks before the start of the experiment), the lyophilized pituitaries were ground with a mortar and pestle, weighed (total amount, 0.273 g), and homogenized with a hand-operated tissue grinder into 35 mL of 0.7% NaCl with NaOH added to adjust the pH to about 9.5. This stock was diluted to four concentrations: 1.3, 2.6, 5.2, and 7.8 μg (dry weight) pituitary material per microliter of saline. These concentrations were then apportioned into the estimated volumes needed on each injection day and frozen at −20° C. A control of the NaCl–NaOH solvent was also apportioned and frozen.

Experimental Procedures

Common carp, captured as young of the year in late September 1976, were held in the laboratory in flowing well water at 10° C until April 1977. Fish, ranging in weight from about 10 to 25 g (mean, 16.9 g), were randomly assigned to four aquaria, 10 fish per aquarium (total, 40 fish). The water temperature was increased from 10 to 35° C over a 2-week period and held at 35° C for an additional 2 weeks (until the start of the experiment). The aquaria contained 22 L of continuously flowing heated well water and were exposed to 10 h/day of fluorescent light. At the start of the experiment the 10 fish in each aquarium were randomly assigned to five treatments, two fish per treatment (total of eight fish per treatment for the four aquaria).

[1] Paper No. 10,963, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

Treatments consisted of four concentrations of pituitary homogenate and the saline control. All fish were cold-branded for identification.

Twice per week all fish were captured, anesthetized with 200 mg/L of MS-222, and injected intraperitoneally just anterior to the pelvic fins with a 27-gauge needle. An injection of 10 μL of a pituitary homogenate per gram of fish, twice per week, resulted in dosages of 26, 52, 104, and 156 μg of pituitary homogenate (dry weight) per gram of fish (wet weight) per week. Fish were injected 1–4 h after daylight; the order of injection was alternated from day to day. Total lengths and weights were taken on the first injection day each week and at termination after 4 weeks. Oregon moist pellets were fed by hand three times per day at the rate of 13% of fish wet weight per day for the first week of the experiment and 15% per day thereafter. Food was withheld for 24 h before the first and last weighings. At the end of the experiment all fish were killed with MS-222 and dried to a constant weight (at 110° C) for determination of percentage moisture.

Data Analysis

Condition factor was calculated as described by Adelman (1977) except that the slope of the length-weight regression in the present study was 3.11. Analysis of variance in a randomized block design and the Student-Newman-Kuels multiple range test were used to examine differences in percentage gain in length, weight, or percentage water due to the treatments. A square-root transformation of percentage gain in length and an arcsin transformation of percentage gain in weight were used to meet the assumption of normality (Steel and Torrie 1960).

Results

Injections of common carp pituitary material enhanced the growth rate of common carp with a dose-related difference in mean percentage increase in both length and weight after 4 weeks (Fig. 1). Saline-injected fish gained significantly less weight and length than fish receiving all other treatments ($P < 0.05$), and fish injected with the lowest pituitary dosage (26 μg/g) gained significantly less length than fish injected with the highest dosage (156 μg/g). None of the other groups of fish grew at significantly different rates from each other ($P > 0.05$). The dose-response curve for length increase was essentially linear through the three lowest pituitary dosages, but rapidly approached an asymptote between the two highest dosages. The curve for weight gain was not asymptotic and was approximately linear over the three highest dosages (Fig. 2).

The condition factor of all fish increased slightly during the experiment, with the greatest increase among fish injected with 26 and 52 μg/g and the smallest increase among the control fish (Table 1). Percentage moisture seemed to increase with increased pituitary dosage, except that injections of 104 μg/g resulted in about the same percentage moisture as the controls (Table 1). Only fish in the control and highest dosage (153 μg/g) were significantly different from each other ($P < 0.05$). Three fish died during the experiment—one each in the control, 104, and 156 μg/g treatment groups.

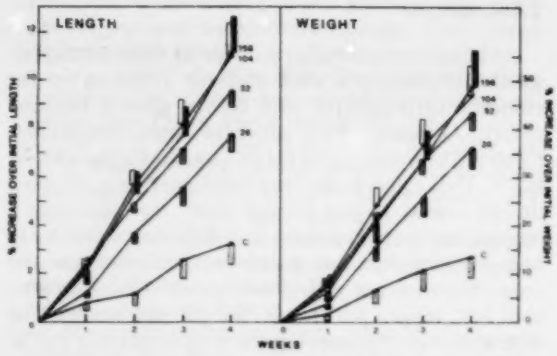

Fig. 1. Percentage gain in body length (total) and wet wieght for groups of common carp injected with different amounts of lyophilized common carp pituitary homogenate. Numbers to the right of each curve give the dosage as micrograms of pituitary per gram of fish per week. Control fish (C) were injected with saline. Bars represent plus or minus one standard error.

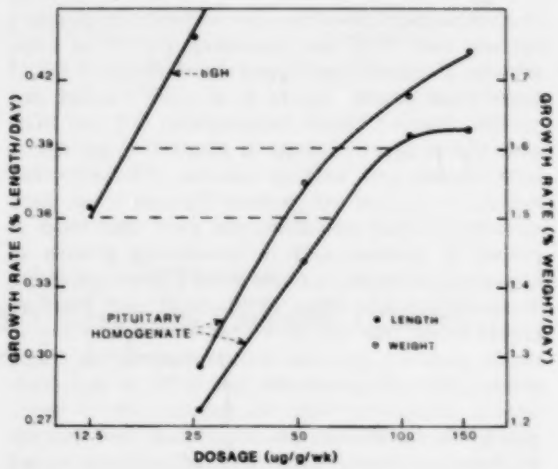

Fig. 2. Dose-response curves for groups of common carp injected with common carp pituitary homogenate or bovine hormone (bGH). The curve for bGH is a segment of the linear dose-response curve from Adelman (1977). The dashed lines indicate dosages of bGH and pituitary homogenate resulting in the same growth rate (in length only) over the linear portion of the dose-response curve.

Discussion

Although conducted at a different time, an experiment determining a dose-response relationship for common carp injected with bovine growth homone (bGH; Adelman 1977) provides some comparison between the potencies of the pituitary material and the bGH. That experiment was conducted in a physical design similar to the present study, but the experimental fish were captured in a different water body and, probably because of their smaller initial size, the controls grew at a slightly greater rate (0.17% compared to 0.12% length per day in the present study). The dose-response relationship for length increase due to bGH was linear throughout the dosage range tested (12.5–100 μg/g per week) and was nearly parallel with the curve for the pituitary material (Fig. 2). The potency of bGH was from 5.0 to 5.4 times greater per unit weight than that of the pituitary material over the linear portion of the pituitary material dose-response curve, where the growth rates coincided.

Higgs et al. (1978) compared the effectiveness of chinook salmon (*Oncorhynchus tshawytscha*) pituitary extracts and bGH for promoting growth of coho salmon (*O. kisutch*) and found the bGH to be 5.4 to 12 times more potent. Clarke et al. (1977) found that purified tilapia (*Tilapia mossambica*) GH and bGH were about equally potent in stimulating growth in both tilapia and sockeye salmon (*Oncorhynchus nerka*). A purified polypeptide fraction from chum salmon (*O. keta*) pituitaries was more than twice as potent as porcine GH in promoting growth of hypophysectomized rainbow trout (*Salmo gairdneri*; Komourdjian and Idler 1979). Swift and Pickford (1965) found that the growth-promoting potency of perch pituitary material varied considerably with season. Since the pituitaries used in the present study were a composite from 10 months over 2 years, potency was probably an average value—less than the maximum attainable if the month of peak activity had been known and selected. Although other pituitary hormones may have influenced the anabolic effect of the common carp pituitary homogenate (Donaldson et al. 1979), the major anabolic activity of pituitary material from chum salmon appears to occur in only a small quantity of a fairly specific fraction (Komourdjian and Idler 1979). Although possibly coincidental, the parallel nature of the dose-response curves of bGH and pituitary material may suggest that a GH-like fraction was primarily responsible for the increase in length due to the homogenate.

Administration of GH to intact salmonids has typically resulted in a decreased condition factor (Donaldson et al. 1979), but with common carp, injections of fish pituitary material (present study) or bGH (Adelman 1977) resulted in an increased condition factor. The increase in condition was clearly dose-dependent with bGH but not with common carp pituitary material. The increased condition factor demonstrated was not simply due to water content since percentage moisture did not correspond to changes in condition factor (Table 1). Komourdjian et al. (1978) suggested that increases in condition factors may result from extrapituitary factors, such as the increasing tameness of the fish resulting from frequent handling.

Although it is possible to enhance fish growth by injections of common carp pituitaries, the impracticality of injecting large numbers of fish makes use of the procedure unlikely except for experimental purposes. Furthermore, the purified bGH was considerably more potent than the unpurified pituitary homogenate and would thus be preferable. Other more practical routes of administration, such as by ingestion or uptake from the water, are of uncertain feasibility (Donaldson et al. 1979).

Acknowledgments

The present study was supported by a grant from the University of Minnesota Agricultural Experiment Station MIN-17-75. I thank James E. Luey for his assistance.

Table 1. Mean condition factor (± SE) and mean percentage moisture (± SE) of common carp injected with various amounts (micrograms per gram per week) of lyophilized common carp pituitary material. Control fish received injections of saline.

	Treatment (μg lyophilized pituitary material per gram wet weight of fish per week)				
Measurement	Control	26	52	104	156
Condition factor					
Initial	1.12 ± 0.02	1.10 ± 0.01	1.07 ± 0.02	1.09 ± 0.02	1.07 ± 0.02
Final	1.16 ± 0.02	1.19 ± 0.02	1.16 ± 0.02	1.15 ± 0.02	1.13 ± 0.04
Difference	+ 0.04	+ 0.09	+ 0.09	+ 0.06	+ 0.06
Percentage water	71.83 ± 0.30	72.09 ± 0.48	72.80 ± 0.37	71.84 ± 0.34	73.34 ± 0.25

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Accepted 24 September 1981

Effects of Wescodyne and Malachite Green on Parasitic Ciliates of Juvenile American Lobsters

Peritrich protozoans have been reported from the external surfaces of numerous aquatic animals (Hyman 1940; Kudo 1966), including the eggs and larvae of the American lobster, *Homarus americanus* (Fisher et al. 1978). During a nutrition study involving juvenile American lobsters, it was noted that many of the animals were infected with *Vorticella*, a species of stalked ciliated protozoa. These epibionts are believed to serve as useful indicators of organic water pollution (Antipa 1977; Henebry and Ridgeway 1979) and at first were not thought to be harmful to the lobsters. Results of the nutrition study, however, suggest that their presence may actually facilitate infections by other microorganisms. Moreover, since the organisms are often attached to gill structures, it is suspected that they may also interfere with respiration of the lobsters.

Two biocides were tested to determine if and how we could eliminate the protozoans. The agents used were malachite green, a fungicide and parasiticide, and Wescodyne (West Chemical Products Ltd., Montreal, Canada), a liquid detergent whose active ingredient is iodine. Malachite green has successfully been used to treat lobsters infected with the fungus *Lagenidium* (Fisher et al. 1976*a*) and may be useful as a general chemotherapeutant against other microbial epibionts (Fisher 1977). Wescodyne is routinely employed as a disinfectant by scientists engaged in lobster aquaculture on the east coast of Canada (personal communications with D. Aiken, Fisheries and Oceans, Canada, St. Andrews, New Brunswick; J. Castell, Fisheries and Oceans Canada, Halifax, Nova Scotia; and E. Mason, Marine Lobster Farms, Prince Edward Island).

In an experiment consisting of two replicates, fourth-stage juvenile lobsters were distributed among 8 sample groups, 5 animals per group (total 80 lobsters) for a period of 18 days. Lobsters were housed in pyrex basins, 13 x 22 x 7 cm (5 lobsters per basin,

total: 16 basins) which were filled with 1,000 mL of sterile synthetic sea water. The water was continuously oxygenated by air stones; plastic caps, 1.5 cm in diameter, were placed on the bottom of the basins to serve as shelters for the animals. Lobsters were fed *Artemia salina* daily. Every 3 days each group of lobsters (16 groups) was removed from the pyrex dishes with a fine-mesh net and dipped into 250-mL glass staining jars, each jar containing one of a series of solutions of malachite green (5, 10, and 20 mg/L) or Wescodyne (250, 300, 500, and 1,000 mg/L) or in a control solution (sterile sea water) for 15 min/treatment, for a total of 5 treatments.

Lobsters were examined under a stereomicroscope 1 day after each treatment for the presence of protozoans and for signs of toxicity. The cuticle, gills, and appendages of all the animals were examined before the first treatment and, depending on the degree of ciliate infection, each lobster was assigned a numerical value, ranging from 0 to 3 (0 = no visible infection; 1 = 1-20% of total surface covered; 2 = 21-50% covered; and 3 = more than 50%). The initial average infection level was determined for each group of 5 animals and these figures were used to compare infection levels after each exposure period according to the following formula:

Relative change in the degree of infection	=	Degree of infection after each exposure	−	Degree of infection before initial exposure

None of the treatments eliminated the ciliate populations.

An abrupt drop in the infection levels and an overall retardation of reinfestation was noted among treated lobsters, compared with those immersed in the control solution (Figs. 1, 2). This change was observed immediately after the animals molted and coincided with the time that lobsters were exposed to the treatments for the first or second time. Some mortalities that occurred at this time were attributed to cannibalism. Although no definite statement can be made, the lobsters treated with malachite green apparently experienced a more pronounced drop in parasitic infestation and slower rate of reinfestation than those treated with Wescodyne. An exception was replica 2, in which lobsters were treated with 250 mg/L of Wescodyne. This test was inconsistent with all other test results in that none of the other iodine treatments proved more effective than did the malachite green treatments.

Neither malachite green nor Wescodyne completely eliminated the *Vorticella* populations on juvenile lobsters. Data from the present study suggest that the concentrations of Wescodyne (100–1,000 mg/L), routinely employed in some of the research laboratories in eastern Canada, may be too low to destroy such organisms. Furthermore, even though malachite green

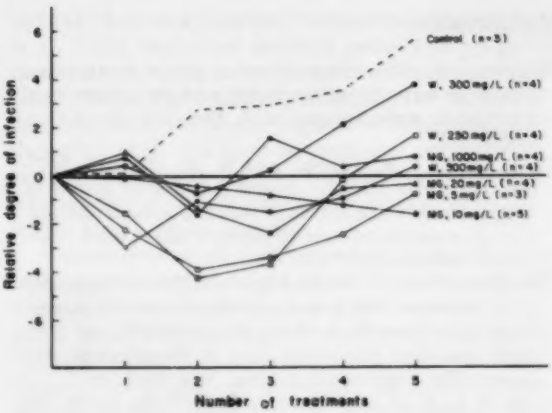

Fig. 1. Relative changes in the degrees of ciliate infection for lobsters exposed to various treatments with malachite green (MG) or Wescodyne (W) at 3-day intervals in replica 1. (Five animals per treatment; n=number of survivors at the end of the experiment).

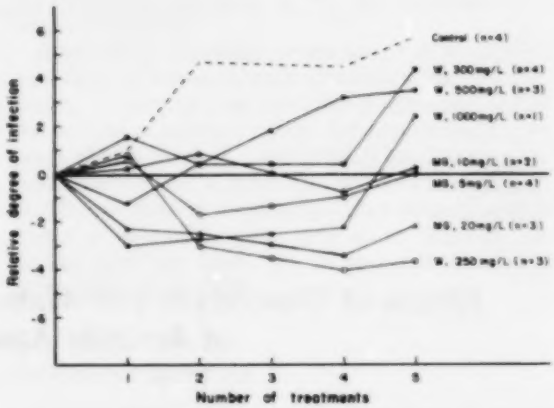

Fig. 2. Relative changes in the degrees of ciliate infection for lobsters exposed to various treatments with malachite green (MG) or Wescodyne (W) at 3-day intervals in replica 2. Five animals per treatment; n = number of survivors at the end of the experiment).

solutions of 20 mg/L represented the upper tolerance limits of lobsters in studies of larvae exposed for 8 min (Fisher et al. 1976*b*), results of the present study suggest that this concentration has no detrimental effects on the protozoan or their hosts, when lobsters are exposed for as long as 15 min.

Future investigations concerning the potential suitability of Wescodyne and malachite green, as biocidal agents for the treatment of juvenile lobsters, will require appropriate toxicity studies with both lobsters and protozoans. Although neither of these agents is

now registered for use in commercial fisheries in the United States, their importance for experimental application should certainly be assessed.

Acknowledgments

Financial assistance provided by the Fisheries and Oceans Subventions Program (Grant 5427-1) is gratefully acknowledged and I thank Denise Doucet for technical assistance.

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Accepted 16 December 1981

Technique for Photographing Small Fish

A variety of techniques has been developed for photographing small live fish (Cruise and Newman 1973: 22–55; Kinne 1962: 139–159; Thomas 1978*a*, *b*). Photographs of fish have been used for many purposes, including estimating weights (Christensen et al. 1977; Hawkes 1975) and lengths (Martin 1967). The recent development of a fiber-optic light-sensing device, the Vivitar MFS-1 Macro Flash Sensor, coupled with the Vivitar 2500 electronic flash, offers an equipment combination that consistently produces high quality photographs of live fish (Fig. 1).

The equipment includes a modified aquarium 41 x 26 x 20 cm, a Pentax H_3V 35-mm single-lens reflex camera, a Vivitar 55-mm 1:2.8 Auto Macro lens, a pistol grip, a Vivitar 2500 electronic flash, and a Vivitary MFS-1 Macro Flash Sensor. The camera and macro lens need not be those specified above. Only Vivitar Corporation, however, produces a commercially available fiber-optic light-sensing device for use with a compatible flash unit and a 35-mm camera.

For taking photographs with this equipment I modified a 1.6-L O'Dell aquarium so that a piece of single-strength window glass (39 x 24 cm) could be placed behind the front glass of the aquarium. Two grooves were filed in the recessed plastic rim at the top of the aquarium about 5 cm behind the front glass

*Fig. 1. A male Ash Meadows pupfish (*Cyprinodon nevadensis mionectes*). The photograph was taken with the equipment described. This exposure was made at f/5.6 on Kodachrome 25.*

(Fig. 2) and the piece of single-strength glass was slipped into the grooves. The angle is such that this piece of glass sits on the joint between the bottom and the front glass of the aquarium, creating a chamber that tapers to 5 cm from the front at the top of the aquarium. The temperature and chemistry of the water should be the same in this aquarium and in the tank where fish are held before being photographed. A flat black background (either card stock or plastic) provided the most suitable background for photographing most species; the background material can be taped to the outside of the back of the aquarium. The photo cell described by Goodbred and Occhiogrosso (1979) can be used instead of a modified aquarium.

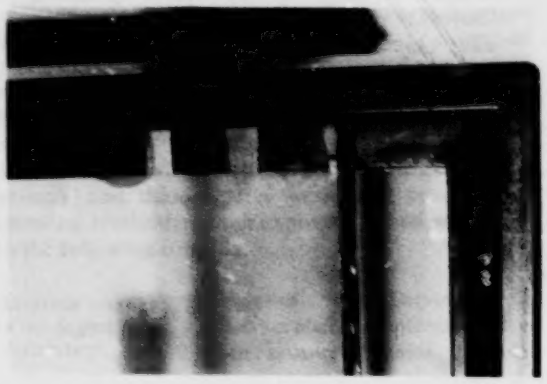

Fig. 2. A top view of an aquarium modified so that a second piece of glass can be inserted behind the front glass of the aquarium.

The photographic equipment (Fig. 3) is assembled as follows:

- Couple the macro lens to the camera body (extension tubes or bellows can be used instead of a macro lens).
- Attach the camera-lens combination to the pistol grip.
- Attach the flash swivel shoe (provided with the MFS-1) to the mounting bracket on the pistol grip.
- Attach Vivitar 2500 flash to the swivel shoe and lock the flash unit to the shoe.
- Using the adjustable swivel shoe, adjust the flash unit so that it points to a spot about 5 cm in front of the lens and the flash unit so that it is at about a 45° angle to the focal plane of the camera (this angle reduces the possibility of flash reflection).
- Pull out the adjustable head on the flash unit until the letter "N" appears in the lower left of the window on top of the flash unit (Fig. 4). This head has three positions (W, N, and T) that correspond to wide-angle, normal, and telephoto settings.
- Connect the PC cord (provided with the MFS-1) to the X connection on the camera body and to the

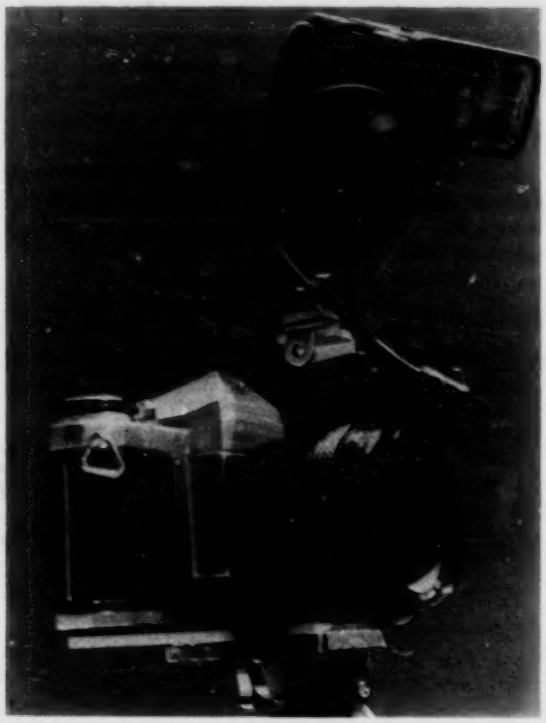

Fig. 3. The MFS-1 Macro Flash Sensor coupled to a Vivitar 2500 flash unit and to camera.

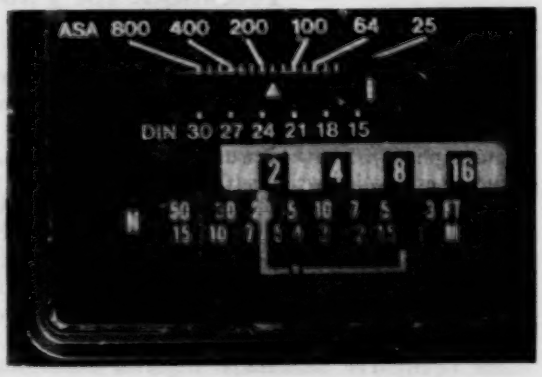

Fig. 4. Settings for film speed, f-stop, and flash mode are visible in the window on top of the Vivitar 2500 flash unit.

receptacle on the lower part of the flash unit.

- Set the mode selector switch on the back of the flash unit to the blue automatic exposure mode.
- Turn the "on-off" switch on the back of the flash unit to the "on" position. A red light on the back of the flash will be illuminated when the capacitor is charged.
- Set the white line on the film-speed selector switch in the window on top of the flash to the proper ASA film speed for the film you are using.
- Set the film-speed indicator on the camera to the proper setting for the film you are using.
- Set the shutter-speed indicator on the camera to the recommended setting for electronic flash (on many cameras this is 1/60 s or is marked with an "x").

MFS-1, the Macro Flash Sensor, is a shielded fiber optic, 30 cm long. The sensor snap couples to the Auto Thyristor Sensor on the front of the flash and the sensor head clips to the top edge of the macro lens (Fig. 5). For optimum results, adjust the sensor head so that it points down at an angle of about 45°. The sensor will then read the light at a point about 5 cm in front of the end of the lens with the MFS-1 in place, and the flash will automatically adjust the light output to obtain a satisfactory exposure.

The manufacturer's instructions state that the lens should be stopped down four stops to obtain a proper exposure with the sensor coupled to the flash. I have found that it is also advisable to bracket one full f-stop on both sides of the adjusted f-stop number. For example, if the automatic exposure guide on top of the flash unit calls for a normal exposure of f/2, the lens should be stopped down to f/8, and exposures should also be made at f/5.6 and f/11.

When coupled with the Vivitar 2500 electronic flash, the MFS-1 Macro Flash Sensor enables consistently reliable, high-quality photographs of small and juvenile fish to be taken under a variety of field and laboratory conditions. The equipment is lightweight and portable, and the flash recharges in less than 1 s. The Vivitar 2500 electronic flash costs about $50; the MFS-1 Macro Flash Sensor, with swivel shoe and PC cord, generally costs about $25; and a pistol grip costs about $25.

Fig. 5. Closeup of the light-sensing head of the MFS-1 clamped to the rim of a macro lens.

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Accepted 30 September 1981

Use of Ozone in a Water Reuse System for Salmonids

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ABSTRACT: A water reuse system is described in which ozone is used in addition to biological filters to remove toxic metabolic wastes from the water. The system functions at a higher rate of efficiency than has been reported for other reuse systems and supports excellent growth of rainbow trout (*Salmo gairdneri*).

Biofiltered aquacultural water reuse systems previously described have required the use of dilution procedures that waste 2 to 20% of the culture water in each cycle (an efficiency of 98 to 80% per pass) to remove toxic metabolic wastes from the system (Burrows and Combs 1968; Mead 1973). This discarding of water reduces the efficiency and economic value of the reuse system, especially when water has been pumped from deep wells and heated or chemically altered. Methods other than, or in addition to, biological filtration should be used to reduce the waste of conditioned culture water.

Water reuse systems have four possible advantages over conventional single-pass systems (Burrows and Combs 1968): (1) reduced cost of fish rearing, resulting from reduced cost for wells and for pumping large volumes of water; (2) reduced cost of heating because the temperature of recirculated water equilibrates with ambient air temperature; (3) increased growth rate of fish due to higher water temperature; and (4) reduced water pollution by hatchery effluents because the water is recycled. By decreasing the waste of conditioned culture water in present reuse systems, this fourth advantage is further enhanced because increased retention time of the biofilter organisms in the system promotes a more nearly complete degradation of organic wastes (Moore and Schroeder 1970), the resultant products having a lower biological oxygen demand (BOD).

Preliminary aquarium studies at the Tunison Laboratory indicated that more than 99% water reuse per pass was possible with salmonids when physicochemical treatments were used to preserve water quality. The most satisfactory treatment was with ozone. The system incorporated an ozone-generated froth condenser, a preozonation-activated carbon denitrifyer, an expanded-bed nitrifying biofilter, and an upflow vertical plate sludge settler. This system had functioned well in an aquarium system and was expanded to operate up to 30 of the 7-L hatchery jars used routinely in nutrition studies at the laboratory.

Pilot-scale Reuse System

The water flow pattern and relation of the components in the system are shown in Fig. 1. The system has a total water capacity of 1650 L, of which 210 L are in the culture units.

The basic design of the upflow vertical plate sludge settler is like that of many commercial units. The settler was constructed with a wood frame and fiberglass-reinforced plastic roofing material for the flocculator plates. The unit measured about 78 x 36 x 15 cm and was separated equally into two sections 39 cm long for ease of removal and cleaning.

Application of froth condensers (protein skimmers) in which ozone is used in the aeration stream has been common in aquarium fish culture (Sander 1967). The condensers incorporate a column of water moving countercurrent to a stream of rising ozonated air bubbles. The bubbles collect and concentrate high-molecular-weight compounds and carry them into collection chambers for removal through a drain located in the bottom of the chamber.

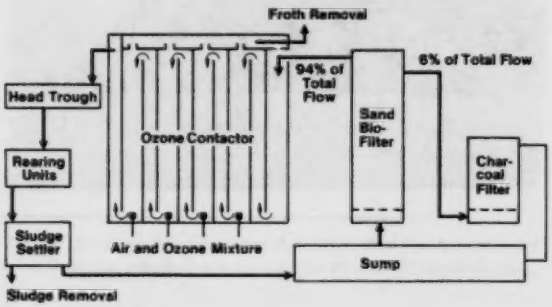

Fig. 1. Schematic diagram showing the relation of various system components and the pattern of water flow (lines with arrows) through the water reuse system. Small solid squares at bottom of ozone contactor represent airstones that introduce the mixture of air and ozone into the columns.

An ozone contactor for the test system was constructed of plexiglass by following the plans of Nebel et al. (1973). The dimensions (122 x 72 x 26 cm) permitted adequate treatment of the 75-L/min flow. The path of the ozonated air through the four chambers of the contactor is shown in Fig. 1. The total airflow for all four chambers (introduced through 24 fretted glass diffusers) was 13 L/min at a pressure of 2.33 kg/cm^2. At the outflow end of the contactor, the treated water cascaded from the top of the contactor into a collecting head trough; this cascading dissipated the residual ozone. The deozonated water was distributed to each culture unit at the rate of about 2.5 L/min.

Since most commercial ozone generators are produced for water disinfection, the generators must be modified to reduce their output of ozone to levels nontoxic to fish. A voltage regulator was added to an ozone generator (Pollution Control Industries model C2P-9C) to reduce the factory-set minimum output of ozone by 50 to 60%. This adaptation is economical and has proved to be durable in our system.

During earlier aquarium studies (unpublished), we found that if a completely colorless reuse water was desired, it was necessary to use an upflow anaerobic denitrifying biofilter with activated carbon, like the one described by Beer et al (1974). Because completely clear water was not necessary for fish culture, we reduced the size of this filter so that only 6% of the water from any one pass was filtered and then returned to the sump. Although this reduction left some color in the water, it adequately kept accumulated nitrate below toxic levels (Westin 1974).

Basic design of the expanded-bed nitrifying biofilter was contributed by Richard A. Speece (Drexel University, personal communication). The filter allowed water to travel up through a diffusion plate at the bottom of the column and to permeate through a bed of quartz sand, where ammonia and nitrites were converted to nitrates, and small particulate matter was trapped and degraded by resident microorganisms. The four filters were constructed from plexiglass cylinders, 15.2 cm in diameter. Each filter contained enough sand to provide an expanded bed of 30.5 cm. This amount of sand provided 13.5 cm^3 of filter medium per liter of volume of the total system.

System Performance

Water in the head trough, having just passed through the ozone columns, was always near oxygen saturation. At near-maximum densities of fish, the oxygen content of the effluent from each jar was less than 60% saturation. Oxygen concentration was further reduced as water passed through the biofilters (Table 1), thus making the efficient aerating characteristics of the ozone columns essential for the metabolism and survival of both fish and biofilter organisms.

Table 1. Average dissolved oxygen (DO) concentrations of the components of the water reuse system.[a]

System component	DO Concentration (mg/L)	
	Inflow	Outflow
Rearing units	7.9	5.9
Sludge settler	6.3	5.9
Sand biofilter	5.9	5.8
Charcoal biofilter	5.8	2.1
Ozone contactor	5.5	7.3
Head trough	8.4	7.9

[a] All values measured at 17.9°C.

Water in the culture units contained a constant 0.02 mg/L of residual ozone as measured by the Hach [PVD] method (Hach 1975). This level apparently had no negative effects on Atlantic salmon (*Salmo salar*), rainbow trout (*S. gairdneri*), or brook trout (*Salvelinus fontinalis*). However, residual ozone above the level of 0.1 mg/L considered safe for salmonids (Benoit and Matlin 1966) occurred when the system was first started and loading densities of fish were low. The amount of ozone generated must be reduced for the smaller load of organic wastes at this time, and then progressively increased as the fish produce more wastes and filter organisms grow. Before the system is loaded with fish, a few fish should be tested to determine if the residual ozone levels are safe. In salmonids, a loss of pigmentation appears to be an indicator of the stress caused by residual ozone. This loss of pigmentation occurs within the first 10-16 h of exposure and allows for adjustments to be made in the ozone level before the test fish suffer permanent gill damage or die. Once the reuse system is in operation, only occasional adjustments of the voltage regulator are required to maintain a safe level of ozone.

The froth removed by the protein skimmers consists of long-chain, high-molecular-weight, soluble carbon compounds made surfactive by the ozonated air stream in the presence of bicarbonate ions, in a process in which manganese and iron ions are catalysts (Eldib 1961). The froth condenses into a concentrated liquid that removes most of the dissolved solids from the system and reduces the BOD (Table 2). The degree of concentration varies with the height of the skimmers above the water surface. The concentration of the condensate is reduced when the skimmer is lowered to the water surface and increased when the skimmer is raised above the surface. The typical composition of this condensate is shown in Table 2. The stream of air bubbles generates a foam, defined by Rubin et al. (1963) as being formed from suspended (insoluble) solids in the water, which also condense in the protein skimmers. The foam is less dense than the froth and appears as a mass of bubbles floating atop the froth.

Table 2. Analysis of solids and biological oxygen demand in condensed liquid waste from the ozone contactor, and the culture water.[a]

Analysis	Liquid waste (mg/L)	Water (mg/L)
Suspended solids	12.50	1.88
Dissolved solids	404.50	411.25
Biological oxygen demand[b]	381.42	111.15

[a] All values are means of two samples.
[b] Samples incubated at 20°C.

(This fraction is included in the analyses presented in Tables 2 and 3).

Water transparencies from 93 to 98% of distilled water were routinely maintained when fingerling rainbow trout were fed Rangen pelleted feeds. A maximum biomass of 37 kg was attained. This resulted in a density of 0.18 kg fish/L or, as it is commonly expressed in fish culture systems, 180 kg/m^3 (11.25 lb/ft^3). The fish appeared healthy throughout the period they were in the reuse system and no pathogenic problems were noted. In an 8-week study conducted with fingerling rainbow trout, both growth and feed: gain ratio in the reuse system exceeded that in the one-pass culture units (Table 4). The improvements were attributable to the influences of (1) warmer water in the reuse system (16–19°C versus 8.6°C for water in our one-pass hatchery system) and (2) the addition of natural food (i.e., nematodes and clumps of bacteria) that was sloughed from the biofilters. Despite the differences in the two rearing systems, this test does provide adequate proof that the reuse system described above will function for fish culture.

Discussion

The small-scale reuse system, suitable for use in salmonid research, described here appears to be feasible and useful. Ozonation of water in a froth-generating column, the nitrifying biofilter, and the use of an activated carbon denitrifying biofilter substituted effectively for the dilution method of removing wastes from a reuse aquaculture system. Eliminating the requirement for post-culture treatment of hatchery effluents by means of advanced physicochemical wastewater treatment adds to the economic attractiveness of closed-cycle aquaculture.

The economic practicality of ozonation has not been fully demonstrated, but the results of the present pilot trial indicate that tertiary water treatments might have a place in water reuse. The prolonged retention time of the aquacultural wastes in a nearly 100% closed system contributes to a more nearly complete degradation by the biofilters. Separation of the biofilters into three distinct treatment environments (i.e., reduction of carbonaceous BOD, reduction of nitrogenous BOD, and denitrification) avoids problems caused by filter clogging and resultant backwashing of poorly degraded solid wastes encountered in previous designs. However, the scale of this system is too small for extrapolation, with certainty, to full-scale, practical aquaculture.

A larger practical unit with various modifications, which has been constructed at the Tunison Laboratory, is being tested and its applicability to full-scale aquaculture is being evaluated.

Table 3. Typical analysis of froth generated by the ozone contactor of the water reuse system.

Component	Value[a]
Protein (%)[b]	17.50
Ash (%, approximate)[b]	30.00
Total alkalinity[c]	75.00
Total hardness	300.00
Turbidity	89.00
Suspended solids	32 15
Dissolved solids	780.75
Calcium	74.00
Copper	0.48
Iron	0.02
Magnesium	115.00
Manganese	0.25
Chloride	75.00
Phosphate	25.00
Silica	65.00
Sulfate	135.00
Ammonia (NH_3)[c]	0.60
Nitrite nitrogen[c]	3.60
Nitrate nitrogen[c]	90.00

[a] Values shown are in milligrams per liter except as otherwise noted; they are typical values, which fluctuate somewhat from day to day.
[b] Based on dry weight of sample.
[c] These values are highly variable and can be affected by the amount of biomass in the system, feeding levels, age of the filters, time since last cleaning, and other factors.

Table 4. Comparative performance of rainbow trout held in two water systems for 8 weeks.[a]

System	Initial weight (g)	Weight gained[b] Grams	Weight gained[b] Percent	Feed:gain[b] (g)
Single-pass system	8.9	14.7x	165x	123x
Pilot ozone system	8.7	16.4y	190y	1.15y
SEM		0.6	2.7	0.02

[a] Fish were held in 7-L hatchery jars (50 fish per jar) and three jars (total of 150 fish) were used in each treatment. No fish died during the study.
[b] Values are the means of three samples. Means in each column followed by different letters are significantly different ($P < 0.05$).

Acknowledgments

We thank Henry Podoliak for his invaluable help throughout the developmental stages of the project.

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Accepted 12 November 1981

Sex-weight Relations in Blue, Channel, and White Catfishes: Implications for Brood Stock Selection

Recent improvements in management of commercial catfish operations have brought rapid economic growth to the southeastern United States aquaculture industry. Although manipulation of the farm pond environment has increased production, little has been done to improve the fish genetically. Immediate areas for improvement may lie in individual selection, retaining the largest individuals for brood stock of channel catfish (*Ictalurus punctatus*), or by the introduction of desirable characteristics from other species through hybridization with white (*I. catus*) or blue (*I. furcatus*) catfish (Dupree et al. 1966; Roberts et al. 1970; Avault 1971; Meyer et al. 1973).

Research in catfish genetics and progress in the commercial industry could be aided by an accelerated selective breeding program. A means to accomplish such a program would be to reduce the generation interval and increase the selection intensity. If the fish were selected at an early age, they could be stocked at low densities in holding ponds and fed ad libitum to facilitate rapid growth and attainment of sexual maturity for future brood stock. Retention of the largest 10% of a channel catfish fingerling population for brood stock was recommended by Meyer et al. (1973). Selection at 6 months would also increase selection intensity since fingerling stocking rate is higher than production stocking rate.

The first convenient time for selection would be when the fish had reached an age of 6 months and a size of 150 mm (30 g). At this time fingerlings would be seined from rearing ponds for stocking at lower densities in production ponds. The next selection might conveniently be made at 18 months when the fish in production ponds are usually harvested.

Beaver et al. (1966) found that channel catfish males grow faster than females and suggested that the largest fish in a normal hatchery population are males. Since fingerling catfish cannot be externally sexed at 6 months, selection on the basis of size alone may result in an inadequate number of females for future brood pairings.

In the present study, we evaluated the relation of sex to the weight-frequency distribution in 6- and 18-month-old channel, white, and blue catfish and the implications such a relation would have for selection of brood stock.

Spawns of channel, white, and blue catfish were produced May 1976 in pens in earthen ponds at the Fisheries Research Unit of the Auburn University Department of Fisheries and Allied Aquacultures, Auburn, Alabama. All eggs were hatched in paddlewheel troughs and sac fry were stocked within 48 h of hatching. Four to eight spawns of each species were stocked in each of two 0.04-ha ponds at 150,000/ha.

Fry were fed 36% protein catfish feed at the rate of 3% of body weight 6 days per week. From November 1976 to March 1977 rations were reduced to a maintenance level of about 1% of body weight fed every other day.

Fingerling rearing ponds were drained in March 1977 and stocks of each species from the two replicate ponds were pooled and restocked into three 0.04-ha ponds at a density of 7,500/ha. Fish were fed 3% of body weight per day, on the basis of mean weights estimated from monthly seine samples during the production season. Ponds were harvested in October 1977, 18 months after the fish were hatched.

At 6 and 18 months, 100 fish of each species were randomly selected from the pond populations. Each fish was weighed and sexed. The aceto-carmine squash method (Guerrero and Shelton 1974) for sexing juvenile fishes was used when the fish were 6 months old. Gonads were removed from the fish with forceps and placed on a glass slide. A few drops of aceto-carmine solution were added and the tissue was squashed with a cover slip. Mounts were examined under a compound microscope at a magnification of 100x. Oocytes were easily seen in ovarian tissue, whereas testicular tissue appeared stringy. At 18 months fish were sexed on the basis of external characteristics (Meyer et al. 1973).

The weight-frequency distribution for channel, white, and blue catfish was determined from random samples of each species (Table 1). The range of values for channel and white catfish males was larger than that for the channel and white catfish females, but was similar for blue catfish males and females (Table 1).

Table 1. Weight-frequency distributions of male (M) and female (F) 6- and 18- month-old channel, white, and blue catfish.

Weight (g)	Channel		White		Blue	
	M	F	M	F	M	F
Age, 6 months						
10–19	4	6	5	7	10	17
20–29	6	13	13	12	22	25
30–39	17	7	27	23	5	7
40–49	8	10	14	3	1	0
50–59	13	7	1	0	—	—
60–69	2	1	1	0	—	—
70–79	3	1	—	—	—	—
80–89	0	0	—	—	—	—
90–99	1	0	—	—	—	—
Age, 18 months						
100–199	1	2	7	12	—	—
200–299	3	2	8	19	5	9
300–399	1	1	8	18	26	30
400–499	3	10	7	9	11	13
500–599	9	8	9	1	3	1
600–699	5	11	1	0	1	1
700–799	3	7	1	0	—	—
800–899	9	7	—	—	—	—
900–999	10	1	—	—	—	—
1,000–1,099	2	0	—	—	—	—
1,100–1,199	2	0	—	—	—	—
1,200–1,299	2	0	—	—	—	—

In samples of channel and white catfish, males had larger mean weights and higher maximum weight values than females (Tables 1, 2). Sex ratios for each group did not deviate greatly from 1:1 in all three species. However, there were indications of significantly fewer females in the largest group of channel and white catfish at 6 and 18 months, confirming the research of Beaver et al. (1966) for channel catfish.

The sex ratio of the largest 10% of 6- and 18-month-

Table 2. Relation of sex to the weight-frequency distribution of 6- and 18-month-old channel, white, and blue catfish (M = male, F = female).

Species and age	Sample size		M:F Ratio	Weight (g)				Heaviest 10% of sample (no.)		
				M		F				
	M	F		Avg.	Max.	Avg.	Max.	M	F	"Heavy" M[a]
At 6 months										
Channel	55	45	1.2:1	40	92	34	70	7	3	3
White	55	45	1.2:1	37	64	33	47	9	1	8
Blue	38	49	1:1.3	27	43	25	42	5	4	1
At 18 months										
Channel	51	49	1.04:1	765	1243	593	907	10	0	15
White	41	59	1:1.4	275	779	290	516	9	1	9
Blue	46	54	1:1.2	386	666	354	608	5	5	1

[a] Number of males heavier than the heaviest female in the sample.

old channel catfish sample was 7 males to 3 females and 10 males to 0 females, respectively (Table 2). Among the 18-month-old fish, 15 males (15% of the total sample) were heavier than the heaviest female. The largest 10% of the 6- and 18-month-old white catfish consisted of 9 males and only 1 female (Table 2). At 6 and 18 months, blue catfish had equal numbers of males and females in the largest 10% of the population (Table 2); both sexes grew at the same rate.

Findings of the present study could be of considerable significance to hatchery managers and commercial farmers with an interest in genetic improvement of catfish growth rate through individual selection. The program of retaining the largest 10% of a channel catfish population for brood stock recommended by Meyer et al. (1973) would have resulted in all-male broodstock populations if applied to the populations of channel and white catfish in the present study. Similarly, in selecting brood fish from the present lot of blue catfish, simply grading for the largest fish would have yielded equal numbers of males and females. However, when selecting channel or white catfish in such populations, care is required to find the largest females. To find the largest 10% of channel catfish females, the largest 30% of the 6- and 18-month-old fish must be graded and sexed and for the white catfish the largest 30-50% of the 6- and 18-month-old fish must be graded and sexed. (Table 1).

Accepted 16 December 1981

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Device for Injecting Preservative into the Stomach of Fish

Examination of the stomach contents of fish is necessary to describe their food habits. To assure an accurate description of a fish's diet, it is imperative that digestion be stopped or the stomach contents removed and preserved immediately after capture. This preserves recently eaten food items in an identifiable condition.

Researchers have used a number of techniques for collecting identifiable fish stomach contents. Giles (1980) and Van Den Avyle and Roussel (1980) used stomach pumps. Needham and Needham (1962) advised slitting the body cavity and placing the fish in preservative; Terry (1977) injected preservative into the stomach of fish, using a hypodermic syringe. We have used a hypodermic syringe, but found it cumbersome when processing a large number and variety of fish species in the field. We also had problems with breakage, spillage, and blockage. We therefore designed a device for injecting preservative into fish stomachs that is easier to use than a hypodermic syringe.

Our device was made from a 473-mL (16 oz) plastic plant sprayer with a minor modification (Fig. 1). The nozzle orifice was enlarged to permit the casing of the

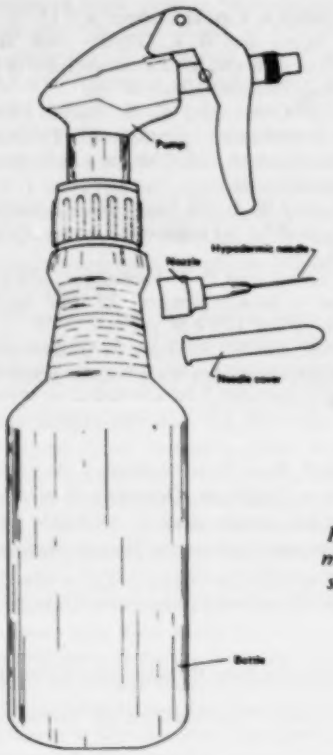

Fig. 1. Plant sprayer modified for use as a stomach injector for fish.

hypodermic needle to pass through the orifice, stopped by the lip on the base of the needle which forms a seal. The needle is held in place by screwing the nozzle onto the pump. The needle cover can be placed over the needle when the injector is not in use. Disposable hypodermic needles are available in a variety of sizes and can be selected according to the size of fish. We found that a 23-gauge needle worked well for fish 60–600 mm long.

Immediately after capture, 10% buffered Formalin was injected through the body wall into the stomach cavity of the fish; care was taken not to evert the stomachs by hydraulic pressure. Later, in the laboratory, injected fish were measured and their stomachs were removed and placed in ethyl alcohol. Formalin does affect the lengths of fish (Parker 1963), so appropriate adjustments should be made.

The advantages of the injector are that it is inexpensive (less than $3) and almost unbreakable; it also has a large capacity and is easy to assemble and repair (i.e., to replace dull or bent needles) in the field. We have processed more than 10,000 fish with this technique and have found the injector efficient and effective for injecting preservative into the stomachs of fish.

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Accepted 16 December 1981

Artificial Turf as a Substrate for Incubating Lake Trout Eggs on Reefs in Lake Superior

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ABSTRACT: In October 1980, more than 50,000 eggs of lake trout (*Salvelinus namaycush*) were placed between layers of artificial turf, 30 x 90 cm. These "sandwiches" were suspended from plastic jugs above the bottom at Devils Island Reef, Lake Superior, and anchored to pieces of railroad track. The total material cost for each sandwich was $45. When they were retrieved from the reef in May 1981, about 78% of the eggs had apparently hatched and most sac fry had left the sandwiches.

In the middle to late 1950's, the sea lamprey (*Petromyzon marinus*) devastated the fish stocks of Lake Superior. Many species increased after lamprey control took effect; however, stocks of lake trout (*Salvelinus namaycush*) have only slowly recovered in most of Lake Superior and the other Great Lakes (Smith and Tibbles 1980). Massive plantings of yearling lake trout in Lake Superior in 1960–70 were not overly effective in reestablishing the populations that used historical spawning reefs. There is considerable evidence that in the past lake trout have returned to their natal reef to spawn (Eschmeyer et al. 1953; Eschmeyer 1955; Martin 1957; Loftus 1958; Rahrer 1968; Swanson 1974). The prime reason for the failure of lake trout stocked in Lake Superior to spawn in these areas has been assumed to be the lack of homing instinct. Even fall fingerling and yearling lake trout stocked directly on these reefs have not demonstrated a strong tendency to home to the reefs after they mature. It has been speculated that lake trout imprint very early in life to the reef where they were hatched (Horrall 1981). If so, the seeding of eggs on these reefs should insure that on reaching maturity, the survivors would home to their natal reef to spawn.

I describe an artificial substrate for egg incubation to be placed directly on spawning reefs and present information on egg survival with the artificial substrate from an experiment conducted on Devils Island Reef in Lake Superior.

Materials and Methods

Artificial turf "sandwiches" were constructed of Monsanto C-4 Astro turf without cloth backing. Two mats (30 x 90 cm), placed turf side to turf side, composed one layer. Seven such layers were placed together in a pine wood frame and strapped together with plastic banding tape by using a dymax tensioner (Signode Company, Chicago, Illinois). The sandwiches were attached to 90-cm lengths of railroad track by two 75-cm lengths of chain 6 mm in diameter (Fig. 1). Sturdy plastic 3.8-L jugs were tied to both ends of the sandwich with 45-cm lengths of rope. The sandwiches were placed on the reef bottom by scuba divers at 13.5 m and the railroad track anchors were attached in series with 6.4-mm cable. The plastic jugs were filled with air from the divers' regulators to give added buoyancy to the sandwiches. The total material cost for each seven-layer sandwich was $45.

Lake trout eggs were sandwiched between the layers of artificial turf; a similar technique had previously been used to incubate eggs of pink salmon (*Oncorhynchus gorbuscha*) in a hatchery (Bailey and Taylor 1954). On 22 October 1980, wild lake trout eggs were taken from the spawning population on Gull Island

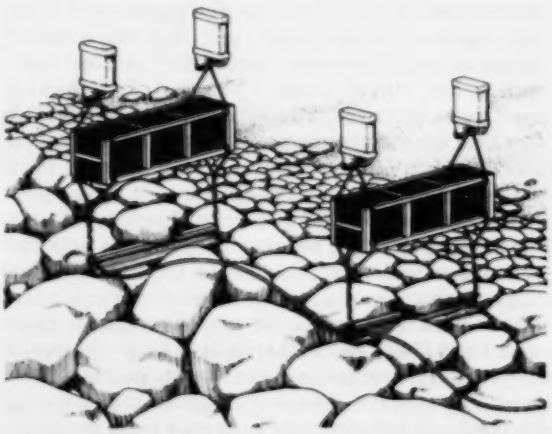

Fig. 1. Placement of artificial turf sandwiches on a spawning reef.

Reef, located just east of the Apostle Islands in Lake Superior, and transported to the Bayfield (Wisconsin) hatchery for placement in the artificial turf sandwiches. The mats were floated in a hatchery raceway while the water-hardened eggs were poured onto the mats (Fig. 2). An attempt was made to avoid clumping the eggs. The eggs were neither treated to control fungus nor picked over to remove dead or dying eggs. Three different quantities of eggs were used in the sandwiches: two sandwiches had 2,000 eggs per layer, one had 1,600 per layer, and two had 1,200 per layer (total, 52,400 eggs). Five sandwiches (four seven-layer and one four-layer) were packaged and placed in 8.3°C water in a hatchery raceway overnight. On the morning of 23 October 1980 the sandwiches were anchored on Devils Island Reef, on the northern fringe of the Apostle Islands in Lake Superior. During transportation to the reef, the sandwiches were held in a cattle-watering tank covered with canvas, and lake water was occasionally poured over them.

Two separate single layers were held in the hatchery at a constant 8.9°C to evaluate sac fry escapement. Each layer held 1,500 eyed eggs that had been treated with malachite green and had been picked over to remove dead eggs.

Results

After 3 months, the sac fry had ceased to leave the layers held in the hatchery. Examination of the two layers revealed 128 dead eggs in the mats; no dead sac fry were observed. Overall hatch and escape for these two layers was 96%.

On 7 May 1981 the sandwiches were removed from the lake and examined for wear and egg survival. Only one sandwich showed wear, and that was a minor scrape on one of the wood frames. One railroad track section had moved about 1.5-m, but the sandwich itself was unaffected. The sandwiches survived their first field test mainly because of their ability to flex and sway like a sea-fan in reaction to the rigorous wave action and currents of Lake Superior.

Egg survival of 79% was estimated by counting the dead eggs remaining between the layers of artificial turf and subtracting this number from the number originally known to be present. Survival was apparently higher in the layers with 2,000 eggs (83%) than in those with 1,600 (74%) and 1,200 (73%). A large number of large sac fry were still present but few dead fry were seen. The scarcity of dead fry suggests that if an egg hatched, the fry were able to escape from the sandwiches. Shipboard facilities did not permit the extraction and retention of live sac fry from the individual layers. Although I was unable to count the live sac fry, a conservative estimate of hatch and escape of fry from the sandwiches was 60–70%.

Fig. 2. Lake trout eggs on an artificial turf mat. The coin is a quarter (U.S.).

Discussion

In comparison with results from direct deposition of eggs on a reef, the artificial turf technique probably reduced predation and eliminated water-hardening as a factor in locking the eggs in the substrate. Reef-reared eggs are incubated at proper water temperatures and pressures, which cannot be duplicated at a hatchery. I suspect that the egg mortality greater than that in a hatchery (usually about 15%) was due to clumping of eggs when they were placed in the mats. If one or two eggs die in a clump, a fungus might infect the adjacent viable eggs.

The benefits of successful lake trout egg seeding on non-producing historical spawning reefs are twofold. The clearest benefit would be the return of mature spawners developed from surviving eggs, due to imprinting. Studies by Swanson and Swedberg (1980) indicated that the return rate of spawning fish (from eggs) on Gull Island Reef was 0.18%. (This return occurred in the presence of an annual fishing mortality rate of only 7.3% due to the protection of a fish refuge.) If the rate to maturity of sandwiched eggs at Devils Island Reef were similar, each sandwich (7 layers with 2,000 eggs per layer; total, 14,000 eggs) could produce about 25 homing spawners. About 6 of these would be expected to be females, since females constitute about 25% of the spawning population on Gull Island Reef (Swanson 1974). At an estimated fecundity of 5,000 eggs (Eschmeyer 1964), the females ultimately resulting from each sandwich could be expected to deposit about 30,000 eggs on Devils Island Reef.

The second benefit of hatching lake trout eggs on former lake trout spawning reefs is the possibility that spawning, egg hatching, or the presence of fry may be necessary to attract stocked hatchery lake trout that have matured to suitable sites. Preliminary work being done by the U. S. Fish and Wildlife Service Great Lakes Fishery Laboratories has indicated that spawning lake trout may be attracted to specific sites by the

chemical residue (pheromones) in the fecal material from fry developing on the site (Neil Foster, personal communication). If this is true, mature stocked lake trout may be attracted to the area in which fry from the sandwiches develop.

The initial apparent success of the technique warrants continuation of the project. More fry would undoubtedly escape if the sandwiches were left in the lake until the end of May. This technique may have application for initiating spawning on the deeper lake trout spawning reefs of the Great Lakes, where the sandwiches could probably be lowered from a vessel without the aid of scuba divers.

Acknowledgments

For their aid and advice, I thank Victor Goodlet, Daniel Hahn, Mary Halvorson, Charles Krueger, James Ludack, and Dennis Pratt, Wisconsin Department of Natural Resources; and Ross Horrall, Phillip Keillor, Steven Leatherberry, and Douglas Stamm, University of Wisconsin–Madison.

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Accepted 8 September 1981

Water-stable Diet for Aquatic Invertebrates

Suitable diets are needed for culturing a wide variety of aquatic invertebrates used for assessing physiological effects and potential enviornmental impacts, and to provide food for culturing young fish. Such preparations should support growth and reproduction, should not adversely affect water qu .lity, and should be water stable to accommodate slow and intermittent feeders (Meyers et al. 1970; Meyers and Zein-Eldin 1972). The objective of the present study was to incorporate nutrients into a water-stable matrix that would provide adequate nourishment for aquatic invertebrates for several days.

The diet was prepared in 1 L of water with the following components (amounts in grams): trout chow, 35; dehydrated alfalfa, 15; Pervinal vitamin-mineral tablets (crushed), 4.5; leaves of sugar maple (*Acer saccharum*), 10; and Difco Bacto-agar, 30.

Many invertebrates are detritus feeders whose food often includes leaves. Maple leaves, collected in autumn, were used in this formulation because amphipods preferred this ingredient. Leaves, with their stems removed, were pulverized with a Hobart grinder or Waring blender containing enough water to allow the equipment to function properly. The homogenate was drained through a food strainer, dried at 41° C, and stored at room temperature. The manufacturer's estimated analysis of other ingredients in the preparation are listed in Table 1.

Nutrient ingredients were mixed individually with 1 L of distilled water, heated to 75° C for 5 min, and

Table 1. Ingredients and analyses of invertebrate diet.

Ingredient, components, and units	No. units	Ingredient, components, and units	No. units
Pervinal[a] (per 2.25-g tablet)		Manganese	0.18
Vitamins		Cobalt	0.01
A (IU)	1,300	Iodine	0.005
D_2 (IU)	260	Methionine	15
E (IU)	1.25	Inositol	5
B_1 Thiamine HCl	0.33	Alfalfa[b] (dehydrated)	
B_2 Riboflavin	0.67	Crude protein	17
B_6 Pyridoxine HCl	0.067	Crude fat	1.5
B_{12} activity (mcg)	1	Crude fiber	27
Niacin	2.75	Trout Chow[b]	
d-Pantothenic acid	0.25	Min. crude protein	37.6
Choline	15	Min. crude fat	8
Calcium	114	Max. crude fiber	5
Phosphorous	105	Max. ash	13
Potassium	52	Calcium, actual	1.2
Sodium	42	Phosphorous, actual	1.0
Magnesium	7.3	Salt, actual	0.8
Iron	1.8	Max. added minerals	1.5
Copper	0.275	Min. Vitamin A (IU/kg)	18,000
Zinc	0.275		

[a] In milligrams, except as otherwise noted. Pervinal is a vitamin and mineral supplement for dog food, manufactured by St. Aubrey, Division of 8-in-1 Pet Products, Inc., Brentwood, N.Y.
[b] Percentages, except as otherwise noted.

stirred constantly to ensure a homogeneous mixture. Difco Bacto-agar was then slowly added with constant stirring to prevent lumping of the agar. Temperature was maintained at 75 ± 2° C for 5 min after the agar was added. (Temperatures below 75° C caused the agar to set improperly, and higher temperatures caused separation [layering] of agar and food ingredients.) The resulting mixture was poured into a flat pan (36 x 23 x 5 cm) and placed in a refrigerator at 4° C. The gelled cake was cut into bars of appropriate size to provide food for the invertebrates for several days.

This diet was fed to amphipods (*Gammarus pseudolimnaeus*), snails (*Physa gyrina*), caddisfly nymphs (*Hesperophylax* sp.), and crayfish (*Procambarus* sp. and *Orconectes* sp.). Feeding frequency was based on the length of time it took the organisms to consume the bars. On this diet organisms survived and grew in fiberglass tanks for up to 1 year in flowing water of 12° C. Amphipods and snails reproduced while on the diet. The preparation remained stable in water at 12° C for more than 1 week (slightly less at higher temperatures) and provided a constant source of food for these benthic organisms that feed slowly and intermittently. The diet ingredients may be altered as needed to meet specific requirements of other invertebrates.

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Accepted 16 December 1981

Two Economical Photoperiod Controls for Laboratory Studies

Laboratory studies on the behavior and physiology of fishes often require careful control of environmental variables such as day-length and light intensity. Various devices for controlling duration and intensity of artificial photoperiods are available, but most are either unreliable or too expensive for small projects. We have developed two simple, economical, and highly reliable devices for use in laboratory studies. One device simulates a natural diel light cycle of sunrise-day-sunset-night, and has a provision for adjusting day-length. It has proved useful in studies requiring simulated sunrise and sunset. The second device controls laboratory lights through a series of photocells activated by sunlight to mimic natural day-length. The device is most useful in long-term studies that require simulation of seasonal changes in day-length but not simulation of sunrise and sunset. We describe the construction and operation of both devices.

The diel-cycle simulator consists of a simple double-pole, single-throw 24-h timer mounted on a backboard 20 x 30 cm. Its clock face is fitted with a plexiglass cam 6.4 mm (¼ in.) thick. The cam is bolted to the clock face on the timer and operates a vertical-throw dimmer switch through a push rod and lever arm. It has an inner radius equal to that of the clock face (41 mm), and an outer radius of 52 mm. The "sunrise" and "sunset" ramps on the cam follow a smooth sigmoid curve between the inner and outer radii of the cam (Fig. 1). As the cam is rotated by the timer, a brass push rod 3.2 mm (⅛ in.) in diameter traces the perimeter of the cam. To reduce friction, the end of the push rod is flattened and polished where it rides on the lightly oiled edge of the cam. The push rod is held in position by a machine bolt (7.9 x 19.1 mm; 5/16 x 3/4 in.) that has been drilled to allow passage of the rod through its length and is installed vertically through the top of the timer box. As the cam turns (one revolution in 24 h), the push rod follows the inner radius of the cam (night), advances up the sunrise ramp, traces the outer radius (day), and returns down the sunset ramp to the inner radius. The distance (degrees of arc) between the sunrise ramp and sunset ramp determines day length, and the slope of the ramps determines the duration of sunrise to sunset. To alter day length or the duration of sunrise and sunset, the cams are changed. The cam shown in Fig. 1 produces a day of about 15 h, including a 2-h sunrise and sunset. An example of the changes in light intensity at 1-min intervals on the sunrise cycle of a cam for a 10-h day is shown in Fig. 2.

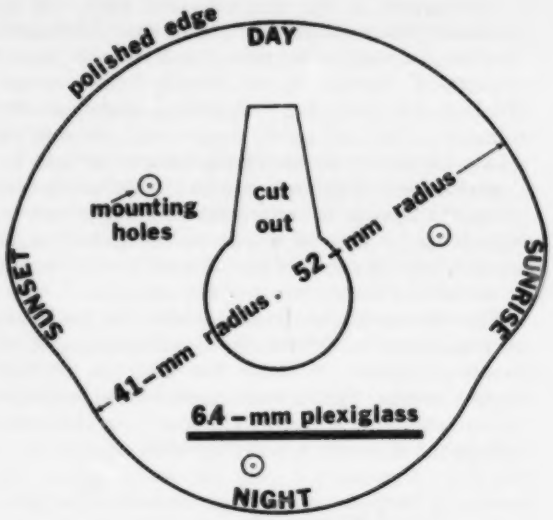

Fig. 1. Configuration and dimensions of the 15-h daylight cam.

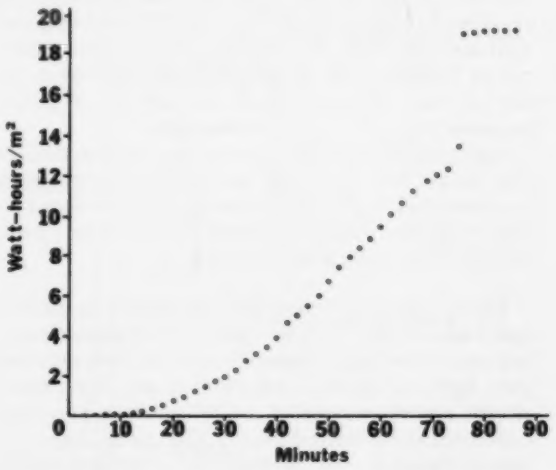

Fig. 2. Changes in light intensity at 2-min intervals during the sunrise cycle of the diel-cycle simulator.

The cams are easily constructed and any natural diel cycle can be simulated by dividing one revolution (360°) of the clock face into the desired components of the cycle. With the cam in Fig. 1 as an example, sunrise and sunset each account for 35° of arc (2 h 20 min), full light for 160° (10 h 40 min), and dark

for 130° (8 h 40 min). The cams are made by scribing the desired dimensions on a plexiglass sheet and carefully sawing around the perimeter. The edges can be smoothed with a fine file and sandpaper.

Movements of the cam-activated push rod are translated into changes in light intensity through a horizontal, spring-loaded plexiglass lever arm attached to a 600-W, vertical-throw rheostat dimmer switch. On the sunrise cycle, the push rod is forced upward by rotation of the cam; on the sunset cycle, the switch is slowly turned off as the spring forces the push rod down the ramp to the inner radius of the rotating cam. When the linkage between push rod and switch has been properly adjusted, the device is relatively maintenance free. Our devices have run for several months consecutively without needing adjustment.

The vertical-throw dimmer switches we use have a click-off, click-on notch at the bottom and top of the rheostat's throw. We have not used the click-off feature, because light intensity is zero at the bottom of the variable voltage throw. The lever arm and switch linkage should be set, however, to click the switch into the full on position during the sunrise cycle. The transition from variable voltage rheostat to full 120-V ac produces about a 30% gain in light intensity (see Fig. 2). The variable voltage output from the dimmer switch is circuited to a standard dual-baseplug receptacle. Aquarium light circuits can be plugged into both receptacles. We have used only one receptacle for experimental lights and have inserted a 7.5-W indicator light into the other. So equipped, the control device can be isolated from the experimental laboratory so workers can determine light intensity at a glance before entering the experimental area.

Materials required for construction of these devices cost about $35 and can be obtained from most hardware stores. Our light controllers are mounted on Dexion, a perforated angle iron, but wood or plastic would also provide a suitable base.

The automatic photoperiod controller consists of a light bulb and two 120-V ac, 1,000-W, 9-A photoelectric switches of the type commonly used to turn outdoor yard lights on at dusk and off at dawn. The photoelectric switch consists of a light-sensitive photocell connected to an electric switch. During daylight, the electric switch is open. At night when no light strikes the photocell, the electric switch is closed and the yard light is illuminated.

We wanted to turn lights on at dawn and off at dusk. To accomplish this, we attached one photoelectric switch to the outside of a light-proof plywood box 24 cm square (Fig. 3) and connected it to a light bulb inside the box. Another photoelectric switch was installed inside the box, opposite the light bulb and

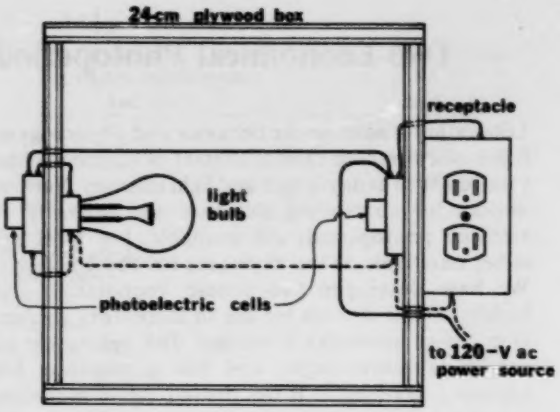

Fig. 3. Schematic diagram of the automatic photoperiod controller.

was connected to a common base plug receptacle located outside the box. At night, no light strikes the photoelectric switch on the outside of the box, and the switch is closed, completing a circuit to the light bulb inside the box. Light from the bulb strikes the photoelectric cell inside the box, which opens the circuit to the outside base plug. Laboratory lights plugged into the receptacle lose power and are turned off. During the day, light strikes the photoelectric switch on the outside of the box and the light bulb is turned off. Consequently, no light strikes the photoelectric switch in the box, the circuit to the base plug receptacle closes, and laboratory lights are turned on.

When the box is placed outdoors, laboratory lights are automatically turned on at dawn and off at dusk. The exact time at which the unit activates or deactivates laboratory lights can be regulated by placing layers of window screen over the outside photocell to decrease the intensity of light striking the photocell.

The device can also be constructed with one photocell and an electric relay. Light striking the photocell leaves the circuit open, closes the relay, and powers laboratory lights. No light on the photocell closes the circuit, opens the relay, and turns laboratory lights off. The device can be constructed easily and economically by either method.

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Accepted 15 December 1981

Saturator System for Generating Toxic Water Solutions for Aquatic Bioassays

Flow-through toxicity tests are becoming more commonplace as environmental awareness increases the need for toxicity data on a variety of chemicals. Chemicals are being tested, or need to be tested, which have low solubility in water but which are soluble enough to have undesirable effects on aquatic organisms. Researchers use the following methods to obtain toxicity data on compounds with low solubility in water: premixing large volumes of water and toxicant, adding toxicant to a carrier solvent and injecting the solution into water (Schimmel et al. 1974; DeFoe 1975), or using saturating devices (Veith and Comstock 1975; Chadwick and Kiigemagi 1968; Gingerich et al. 1979). Use of a saturation device, such as the one described by Veith and Comstock (1975), is desirable because neither large reservoirs of toxicant solution nor additional solvents are required. Their device, however, has definite flow limitations and is complicated to build, operate, and maintain. The objective of the present study was to develop a simplified saturation device with an almost infinite flow capacity.

Soda carbonation cans of the type used in automatic vending machines were employed. These 19-L (5-gal) vessels of stainless steel construction have a large port at the top which allows easy access for cleaning and modification (Fig. 1). The vessel has quick-connect couplers (labeled "in" and "out") for attachment of tubes from a metering pump to the diluter system or between vessels. The out coupling of each vessel has a stainless steel tube running to within a few millimeters of the bottom of the vessel. We calibrated a metering pump, controlled by an adjustable timer, to deliver a desired amount of diluent water into the full saturator can (or cans) during each diluter cycle. When diluent water was added to the saturator an equal volume of saturated solution was forced into the exposure apparatus.

Liquids with higher specific gravity than water were poured into the vessel to a depth of 2–5 cm on the bottom and stirred with a magnetic stirrer slowly enough so that the compound was not emulsified or suspended in the water column. The metering pump was connected to the out coupling on the vessel for these compounds; by this means diluent water could be added to the bottom of the vessel containing the compound and saturated liquid removed near the top of the vessel.

This saturator system functioned equally well for liquids with a specific gravity lower than water. For compounds that floated on the water in the vessel, the pump was connected to the in coupling of the saturator cans; the diluent water could thus be pumped into the top of the can containing the compound and the saturated liquid forced out the bottom. To promote mixing, magnetic stirrers were also used with liquids that floated.

For solid toxicants, a filter (composed of a drilled neoprene stopper and a 30-cm length of 20-mm glass tubing) was installed on the in coupling of the vessel, as shown in Fig. 1. Glass wool was placed in the glass tubing to prevent undissolved compound from being pumped out. This method worked equally well whether the solid compounds sank to the bottom or floated to the top of the water. Solids were stirred rapidly with a magnetic stirrer which made a suspension in the saturator, thus increasing surface area contact with the water.

To test kelthane, a tar-like semiliquid, we first

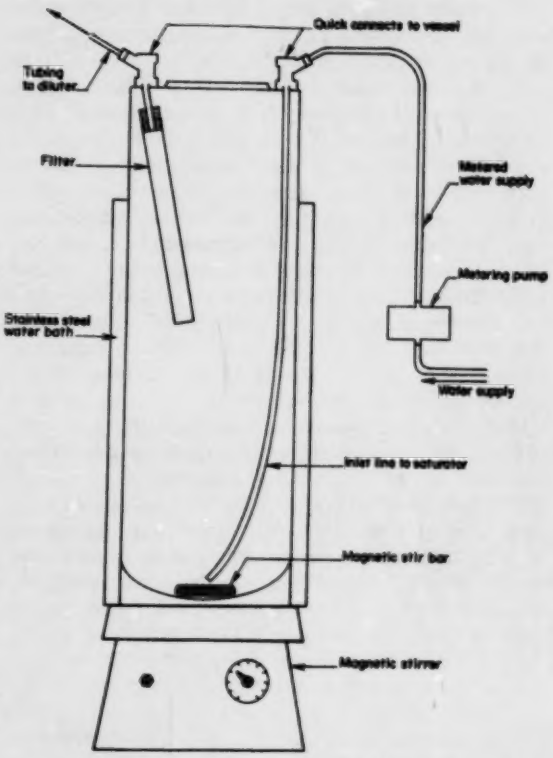

Fig. 1. Cut-away view of the saturator apparatus (carbonation can), showing the water jacket, the metering pump, and the magnetic stirrer and stir bar.

diluted it with acetone to the consistency of heavy paint and applied it with a natural bristle brush to a 10-mesh stainless steel screen that had been formed into a column 10 cm in diameter and 50 cm high. The acetone was allowed to evaporate and the screen column was then placed in the saturation vessel, which was immediately filled with water.

Pesticides with a consistency like honey, such as pydrin and permethrin, were coated on the sides of the vessel. About 20 g of compound were diluted with acetone to the viscosity of water and placed in the vessel, which was tipped to the horizontal position on a steam table. The vessel was then rotated on its horizontal axis until the acetone was completely evaporated. This procedure covered the sides of the vessel with droplets and effectively increased the surface area in contact with the water. The vessel was immediately filled with water to prevent the compound from sagging to the bottom.

The amount of compound added to the vessel varied. If the solubility was low and the toxicity was high, less compound was needed to achieve a lethal concentration. When the compound was fairly soluble, but toxicity was low, larger amounts of compound were added so the toxicant would not be exhausted before the test was completed.

To achieve higher concentrations of toxicant in water the vessels were either heated, or another 19-L vessel was connected. We found that the solubility and rate of dissolution of some compounds could be increased by heating the water in the water jacket (Fig. 1). When the system was heated, the temperature was maintained within ± 1 Celsius degree, and care was taken neither to exceed the degradation temperature of the compound nor to exceed the compound's solubility in water at the test temperature. If saturation was exceeded, the compound would condense and form droplets of compound in the chamber of the diluter to which the supersaturated solution was added. However, better toxicant concentration uniformity was achieved by adding more vessels in line; heat should be used only as a last resort.

The "soda can saturator" has been used successfully to solubilize, in water, chemicals with varying degrees of hydrophobicity. The coefficients of variation of water samples taken from the high concentration exposure tanks and analyzed daily during 96-h acute exposures were as follows: tetrachloroethylene, 15.3%; 1,1',2,2'-tetrachloroethane, 25.3%; pentachloroethane, 5.7%; 1,1',2-trichloroethane, 11.8%; hexachlorobutadiene, 6.1%; 1,1',2-trichloroethylene, 2.3%; 1,2-dichloropropane, 22.1%; 1,2-dichloroethane, 33.2%; hexachloroethane, 26.2%; 1,3-dichloropropane, 2.8%; kelthane, 13.5%; dursban, 8.7%; disulfoton, 17.8%; pydrin, 22.1%; and permethrin, 23.4%. These coefficients of variation also include the normal variation of the dosing apparatus and the variation in the analytical methods used for measuring the compounds. Thus, they underestimate the precision achievable with this saturator. The chemicals tested have been in solid, semiliquid, and liquid phases. Although the saturator has not been tested with chemicals in the gaseous phase it should work with them also, since its original purpose was to dissolve carbon dioxide in soda pop.

Because the system is completely closed, it works well with volatile compounds. This has two distinct advantages: the compound is not lost to the atmosphere, and the work space is not contaminated. These advantages are especially important when heat is applied to increase solubility, since volatility is then also increased.

The use of a metering pump to displace toxicant-laden water with fresh water simplifies the water replacement system. The pump and the water replacement systems can be reused since they are on the influent side of the system and, thus, are not contaminated.

The design and simplicity of the system makes cleaning easy—an important consideration in short-term testing. Since large numbers of chemicals are often used in short periods, frequent and thorough cleaning is necessary to prevent toxicant carry-over from test to test. The stainless steel construction enables the use of solvents, acids, or bases in cleaning, cuts down on breakage, and insures safe handling.

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Accepted 1 September 1981

THE

Progressive Fish-Culturist

A Quarterly for Fishery Biologists and Fish-Culturists

Volume 44 July 1982 Number 3

CONTENTS

Urine Flow Rate in Freshwater Salmonids: A Review. *J. B. Hunn* 119

Collapsible Cage for Holding Fish. *P. Hickley* 125

Effect of Acclimation Time on Nitrite Movement Across the Gill Epithelia of Rainbow Trout: The Role of "Chloride Cells." *S. R. Krous, V. S. Blazer,* and *T. L. Meade* 126

Performance of Rainbow Trout Fed Gelatin-bound Diets of Fish Protein Concentrate or Casein Containing 25 to 45 Percent Herring Oil. *R. O. Kellems* and *R. O. Sinnhuber* 131

"Green Water" Culture of Grass Shrimp in Saltwater Aquaria. *W. W. Falls* 135

Observations of a 4° C Groundwater Source as a Release Site for Atlantic Salmon. *B. Johannesson* 136

Influence of Temperature, Water Hardness, and Stocking Density on MS-222 Response in Three Species of Fish. *J. R. Sylvester* and *L. E. Holland* 138

Prophylactic Treatment of Elvers for *Trichodina* Infestation. *A. G. Eversole* and *D. W. Field* 142

Chamber for Maintaining Adult *Daphnia* and Separating the Young. *P. E. Bertram* and *A. S. Brooks* 144

Techniques for the Capture and Handling of Atlantic Mackerel with Special Reference to the Use of Quinaldine. *T. C. Lambert* 145

Observations on Salinity Tolerance of Striped Bass x White Bass Hybrids in Aquaria. *R. J. Wattendorf* and *P. L. Shafland* 148

Influence of Formalin on Size of Walleye Eggs. *S. L. Serns* 149

Two-stage Collector for Floating Eggs. *R. Pitt* 150

Golden Shiner Virus: Effects of Stocking Density on Incidence of Viral Infection. *T. E. Schwedler* and *J. A. Plumb* 151

Technique for Converting a Beach Seine for Operation by One Person. *S. E. Campana* 152

Incubator Incorporating Air-powered Water Flow for Marine Fish Eggs. *A. E. Giorgi* 154

Exposure of Largemouth Bass to Components of an Osmotic Infiltration Technique: Tolerance and Efficacy. *J. E. Marks* and *D. H. Lewis* 155

First-year Growth and Survival of Bluegill and Black Bullhead Stocked with Largemouth Bass in South Dakota Ponds. *J. J. Shelley* and *T. Modde* 158

Aquarium Airline Tubing Marker for Observing Fish Movements. *R. J. Medved* 160

Retention of Coded Wire Tags in Lake Trout. *D. E. Ostergaard* 162

Urine Flow Rate in Freshwater Salmonids: A Review

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ABSTRACT: Urine flow rate in salmonid fishes was first measured in 1937 and reported to range from 50 to 160 mL/kg per day at 10°C. Later studies on the kidney function of salmonid fishes in fresh water indicated that urine flow rate is influenced by (a) nutrition and disease, (b) environmental temperature and water quality, (c) handling and anesthesia, (d) hypoxic stress and confinement, (e) exposure to certain xenobiotics, and (f) entry into seawater.

Survival of animals in fresh water, teleost fishes in particular, depends on the maintenance of a proper ionic and osmotic gradient between the "interior environment" of the fish and the surrounding water. Maintenance of the gradient usually involves five requirements (Baldwin 1964): (a) possession of a variably permeable boundary membrane (gill epithelium); (b) restriction of this permeable surface to a relatively small area by the presence of a relatively impermeable skin; (c) possession of an excretory organ (kidney) capable of excreting a hypotonic urine and therefore of doing osmotic work; (d) use of salt contained in food; and (e) possession of special cells somewhere on the surface of the fish to absorb salts from the environment and do additional osmotic work that would otherwise fall upon the excreting organ. In the years since these requirements for survival in fresh water were outlined, much progress has been made in developing an understanding of the processes involved with the function of fish skin and gills (Conte 1980; Lahlou 1980). A review of information on kidney function in fishes was published by Hickman and Trump (1969). The present limited review is essentially restricted to studies on urine output from salmonids in fresh water.

History

The first measurements of urine flow rates (UFR) for rainbow trout (*Salmo gairdneri*) were reported by Krogh (1937). Krogh found that in "resting" rainbow trout held at 10°C the UFR varied from 50 to 106 mL/kg per day. No additional measurements were published until Holmes (1961) reported that "stress" could elevate the UFR tenfold over the control levels (75 to 90 mL/kg per day). Although his report was little more than an abstract, Holmes (1961) was the first to note a reduced UFR in trout adapted to seawater. The first extensive urine analysis reported for rainbow trout was that of Fromm (1963). Although Fromm's main concern was nitrogen excretion, he also measured concentrations of Na^+, K^+, Ca^{++}, Cl^-, and osmolality of the urine. Using data on plasma concentrations of these ions and certain assumptions based on work of Holmes and McBean (1963), Fromm estimated the renal tubular absorption of Na^+ and Cl^- as 97 and 96%, respectively.

Since 1963 about one paper per year has been published on renal function in salmonid fishes (Table 1) with some 82% on rainbow trout compared with 7% each for brown trout (*Salmo trutta*) and coho salmon (*Oncorhynchus kisutch*), 4% for lake trout (*Salvelinus namaycush*), and none for other salmonids. Research has progressed during the last 40 years from relatively simple measurement of urine volume and detection of the Cl^- ion in the urine (Krogh 1937) to rather sophisticated attempts to define the role of the kidney in the acid-base balance of trout (Wood and Caldwell 1978) and measurements of hormone actions on single nephron function (Henderson and Brown 1980).

Factors Affecting Urine Flow Rate

Nutrition and Disease

The impact of disease organisms, nutritional deficiencies, and chronic exposure to high levels of "free CO_2" on renal functions of trout have been little investigated.

Krogh (1937) and Holmes (1961) did not give the nutritional status of the trout used in their studies, whereas trout used by Fromm (1963) and Hunn (1969) were from populations routinely fed commercial trout pellets. In other studies, food was withheld from fish for various periods before urine was collected: 1 day (Enomoto 1967), 2 days (Lloyd and Orr 1969), 4 days (McKim 1966), and 1 week (Holmes and Stainer 1966). Cowey et al. (1977) found that a dietary

Table 1. Urine flow rates (UFR) of certain salmonids in fresh water, as reported in the literature.

Species and reference	No. of observations	Fish used		Water temperature (°C)	Collection period (h)	UFR (mL/kg per day)
		Number per test	Average weight (g)			
Rainbow trout						
Krogh (1937)	–	1	170	10	24	59–106
Holmes (1961)	–	–	150–280	10	–	75–90
Fromm (1963)	32	–	138	12–14	24	101
Holmes and Stainer (1966)	–	8–14	–	4–10	20–154	50.7–110.7
McKim (1966)	–	4–16	230–280	10–12	24	9–98
Enomoto (1967)	–	9	127	7–16	18	88.9
Hunn et al. (1968)	1	5	350–515	12	24	81.0–134.4
Hunn (1969)	19	19	167–402	12	4	91.9
Lloyd and Orr (1969)	1	8	–	10.0	16	52.8
	1	5	–	12.5	16	50.4
	1	6	–	15.0	16	64.8
	1	6	–	17.5	16	96.0
	1	4	–	20.0	16	100.8
Hunn and Willford (1970)	30	5	305–660	12	2–12	44.9–215.9
Mayer[a] (1971)	–	20	230–460	17.6	24	74.6
Wood and Randall (1973)	–	8	180–350	14	1–2	100.6
Beyenbach (1974)	77	–	350–600	11–13	–	91.7
Lloyd and Swift (1976)	–	9	–	12–17	–	67.2
Jackson et al. (1977)	–	5	200–1,000	–	–	80.2
Henderson et al. (1978)	–	5	200–1,000	–	–	80.2
Swift (1978)	–	20	–	15	–	81.6
Wood and Caldwell (1978)	–	6	140–301	10–18	4–12	57.6
Allen et al. (1979)	–	4	426	12	12	73.0
Bath and Eddy (1979)	–	4	350	10	–	72
Hoffman and Butler (1979)	5/day	9	297–865	7	–	33.6
Weber et al. (1979)	24	8	100–250	13	1	112.8

Table 1. Continued.

Species and reference	No. of observations	Fish used: Number per test	Fish used: Average weight (g)	Water temperature (°C)	Collection period (h)	UFR (mL/kg per day)
Coho salmon						
Miles (1971)	37	2	1,883–3,176	–	–	43.4
Hunn and Allen (1975)	6	5–7	311	12	4–8	107.5
Brown trout						
Oduleye (1975*a*)	–	–	–	15	24	91.7
Oduleye (1975*b*)	–	6	–	15	24	74.4
Lake trout						
Hammond (1969)	14	5	725–2,700	8	–	26.2–91.0

[a] F. L. Mayer, Columbia National Fishery Research Laboratory, personal communication.

magnesium deficiency can induce nephrocalcinosis (calcium deposits in the kidney) in rainbow trout and more recently, R. R. Smith (personal communication) showed that the type of diet fed to rainbow trout can influence UFR (Table 2).

Indirect evidence provided by Hunn (1964) on bacterial kidney disease in brook trout (*Salvelinus fontinalis*), and by Amend and Smith (1975) on infectious hematopoietic necrosis in rainbow trout, suggested that renal dysfunction was involved in the pathophysiological response of trout to the causal pathogenic organisms. However, no direct measurements of kidney function, including UFR, were made.

Studies by Smart et al. (1979) and Eddy et al. (1979) provide substantial evidence that chronic exposure to high levels of "free CO_2" (12 – 60 mg/L) can also induce nephrocalcinosis in rainbow trout. The percent of the total populations exhibiting gross nephrocalcinosis were 4.8, 9.7, and 45.2 at "free CO_2" concentrations of 12, 34, and 55 mg/L, respectively. Analyses indicated that ureteral deposits were composed mainly of calcium phosphate and fish continued to grow despite severe kidney damage. See Landolt (1975) for a review of calcinosis and associated conditions in fish.

Urine was collected by Eddy et al. (1979), but no values for UFR were reported. The authors, however, stated that they encountered difficulties in collecting urine through catheters from trout exposed to the highest level of "free CO_2" (35 mm Hg P_{CO_2}).

Temperature

Studies of the influence of temperature on UFR in fishes date from the work of Wikgren (1953), who found that in *Petromyzon* the UFR (mL/kg per day) declined with decreasing temperature (359 at 16–18° C; 160 at 7–9° C; and 60 at 2–3° C). Lloyd and Orr (1969) noted an increase in UFR from 52.8 to 100.8 mL/kg per day for rainbow trout when the temperature was raised from 10 to 20° C. Hoffman and Butler (1979) reported a UFR of 33.6 mL/kg per day for rainbow trout at 7° C. Using these data, one can calculate a Q_{10} of about 2, which is more or less consistent with Q_{10} estimates based on oxygen consumption measurements. A similar Q_{10} of 1.9 for permeability to water was determined by Evans (1969), using goldfish (*Carassius auratus*) and the minnow *Phoxinus phoxinus*.

Table 2. Calculated values for urine output by rainbow trout fed various diets while being held in hard water at 15° C.[a]

Diet	No. of fish tested	Urine flow (mL/kg per hour): Mean	Urine flow (mL/kg per hour): ± SD
H-440	19	4.23	1.05
Soybean meal	19	3.30	0.76
Pr 8 G	7	3.34	1.14
Abernathy 19	7	5.15	2.16
Abernathy 18	6	3.28	0.88
Cotton seed meal	8	3.27	0.88
Fish meal (anchovy)	6	5.34	0.72
Fish meal (herring)	5	5.77	0.68
Brewer's yeast	6	3.56	1.54
Soya commercial	5	3.31	1.01
Fish meal (whitefish)	7	3.76	0.55
Wheat middlings	7	3.69	1.35

[a] These values were calculated from data provided by Robert R. Smith (personal communication), U.S. Fish and Wildlife Service, Hagerman Field Station, Hagerman, Idaho.

No literature was found on studies of the effect of fluctuating temperatures within the physiological range for trouts.

Handling and Anesthesia

Holmes (1961) indicated that for short periods after handling or mechanical restraint, the urine output of trout and its chloride concentrations may rise to 10 times the control values — e.g., 75–90 mL/kg per day and 5–12 mM/L. Work by Evans (1969) showed that the handling stress caused an immediate increase in water permeability resulting in diuresis. Handling stress increased water permeability more markedly in large brown trout than in smaller specimens (Evans 1969).

Neither Krogh (1937) nor Holmes (1961) mentioned the use of an anesthetic to quiet trout before handling. Most investigators after 1963 reported using MS-222 at various concentrations. Evans (1969), in studies on water flux across the gills of teleosts, found that anesthetization of goldfish with MS-222 had no effect on water balance in these fish. However, Hunn and Willford (1970) found that rainbow trout anesthetized with MS-222 excreted larger volumes of urine after anesthesia than did those anesthetized with methylpentynol. The higher UFR occurred concurrently with the elimination of MS-222. They also observed that the increased urine output following MS-222 anesthesia was accompanied by an increased concentration of Na^+ in the urine. Hunn et al. (1968) reported that the blood and urine of rainbow trout were cleared of MS-222 in 8 and 24 h, respectively. Further details on the metabolism and excretion of anesthetics by fishes were given by Hunn and Allen (1974).

Catheterization and Confinement

Published descriptions of techniques indicate that trout were subjected to varying degrees of hypoxia during catheterization. Fromm (1963), for example, exposed fish to air during the catheterization process. Both McKim (1966) and Hunn (1969) demonstrated that the urine output of trout was greatly increased after acute hypoxic stress. Also, work by Houston et al. (1969) showed that anesthesia and aortic catheterization caused marked cardiovascular changes and shifts in body fluid composition. This finding indicates that the trauma of surgical procedures can influence the outcome of studies done on renal function if trout are not allowed sufficient recovery time. Therefore, it seems prudent to recommend a recovery period of at least 24 h after catheterization before collecting "normal" urine samples.

Krogh (1937) used a special cage immersed in running tap water to hold trout for urine collection. Holmes (1961) collected urine from trout by using a catheter retained by a harness, but gave no description of the container used for storage of urine during the collection period. Experimental chambers used by recent investigators have been tubes or rectangular boxes made of clear plastic. Further refinements, such as the use of properly placed outlets that allow different parts of the chamber to be flushed, were reported by Post et al. (1965). All chambers described have the disadvantage of confinement, which reduces the activity of fish to less than normal levels.

Studies by Wood and Randall (1973) on the influence of swimming activity of rainbow trout on their ionic balance revealed that, although daily UFR was highly variable, it rose during exercise (resting 100.56 mL/kg vs. active 144.96 mL/kg). The UFR was further elevated during the first few hours of recovery from the exercise (first hour 220.32 mL/kg per day; second hour 137.5 mL/kg per day). The trout were exercised for about 40 min at 14° C.

Studies by Holmes and Stainer (1966) and McKim (1966) showed that daily urine output declines after catheterization and confinement of trout in urine-collecting chambers (Table 3). The reduced urine output is not accompanied by an increase in water content in trout deprived of food. Phillips et al. (1960) showed that body water content increased only 2% in brook trout that had fasted for 2 weeks. The reduction in metabolic rate, resulting from food deprivation or reduced activity that is accompanied by shunting of some blood away from the respiratory lamellae in the gills, may be responsible for a reduced influx of water across the gills.

Table 3. Calculated urine flow rate (UFR) of rainbow trout confined to a urine-collecting chamber from 1 to 10 days at 10–12° C.[a]

Day	No. of fish	UFR (mL/kg per day)	
		Mean	Range
1	16	85	57–98
2	16	58	34–77
3	16	50	36–61
4	6	44	27–58
5	6	37	26–47
6	6	34	27–40
7	6	27	15–38
8	6	27	17–35
9	6	23	9–35
10	6	22	9–31

[a] Values calculated from data provided by J.M. McKim III (personal communication, 1968).

Water Quality

Lloyd and Swift (1976) reported on some of the physiological responses (including UFR) of freshwater

fish that were exposed to low levels of dissolved oxygen and to high levels of carbon dioxide, ammonia, and phenol. Hunn (1969) and Swift and Lloyd (1974) reported that exposure of rainbow trout to hypoxic conditions increased UFR and loss of salts in the urine. Exposure to ambient un-ionized ammonia produced the most dramatic increases in UFR recorded for rainbow trout by Lloyd and Orr (1969). They suggested that this diuresis was caused by an increase in the permeability of the fish to water. These authors further suggested that the ammonia becomes lethal when the permeability is increased to the extent that the influx of water exceeds the maximum sustained rate of urine production (i.e., more than 280 mL/kg per day). However, it is also clear from their work that trout can acclimate to sublethal levels of ammonia.

Work by Oduleye (1975*a*, *b*) and Potts (1980) indicated that environmental (water) levels of Ca^{++} affected the permeability and transepithelial potential of the gills of brown trout. Increased Ca^{++} levels reduced both the water and Na^{+} permeability of the gill membranes. The use of salt to reduce "hauling loss" during and after transportation of salmonids and other fishes is now an established practice. Work by Wedemeyer (1972) established that 75 ppm Ca^{++} or more completely prevented stress-induced hypochloremia in juvenile coho salmon. However, tests were not made with $CaCl_2$ or mixtures of $CaCl_2$ and NaCl to determine which would best accomplish the desired reduction of handling and hauling stress. Normally the total quantity of salts used in freshwater transport should not exceed 0.75%, so that the medium is still hypo-osmotic to the fish. Under these conditions, water would tend to enter fish osmotically and be available for excretion by the kidney.

Excretion of Xenobiotics

Biotransformation and excretion of xenobiotics by fish have been studied by numerous investigators (Khan et al. 1979). Only a few reports included measurements of kidney function, including UFR. Hunn and Allen (1975) observed an increase in UFR in coho salmon exposed to 3-trifluoromethyl-4-nitrophenol (TFM), but the diuresis was not correlated with the pattern of excretion of either free or glucuronide-bound TFM. No such diuresis was noted by Allen et al. (1979) in rainbow trout exposed to 2,5-dichloro-4-nitrosalicylanilide (Bayer 73) by either intraperitoneal (i.p.) injection or bath. Bayer 73 was found in the urine in both the free and conjugated forms. Swift (1978) noted that exposure of rainbow trout to phenol resulted in no increase in UFR and that only free phenol could be detected in the urine. Rainbow trout injected i.p. with CCl_4 at a dose rate of 2.0 mL/kg exhibited a significant drop of UFR (1.4 vs. 4.7 mL/kg per hour), as shown by the work of Weber et al. (1979). Pheifer and Weber (1979), using a dipstick test (Albustix) to detect proteinuria in trout treated with CCl_4, found that only trace amounts (< 80 mg/100 mL) appeared intermittently during the 24 h after treatment.

Adjustment to Seawater

Not all the factors affecting smoltification and migration of salmonids to the sea are known (Wedemeyer et al. 1980). It is known that kidney functions, and UFR in particular, are dramatically changed. Holmes (1961) first noted the reduction in UFR in rainbow trout that accompanied adaptation to seawater. Studies (Hoffman and Butler 1979; Bath and Eddy 1979) have documented the contributions of metabolic rate and of respiratory and ionic regulation to the physiological processes required for fish to survive the transition to seawater.

Henderson and Brown (1980) studied the function of single nephrons (the functional units of the kidney) and indicated that there are three types of nephrons: Type I, functional; Type II, nonfiltering; and Type III, nephrons not perfused with arterial blood. The distribution of the three types differs substantially in rainbow trout adapted to fresh water in comparison with those adapted to seawater. These preliminary studies indicated that the adaptation of trout to seawater is associated with a significant reduction in the number of functional nephrons. Much research remains to be done on hormonal and other factors that regulate the function of the nephron and kidney in fishes.

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Accepted 9 December 1981

Collapsible Cage for Holding Fish

Temporary fish-holding cages for use in shallow water are often invaluable to people engaged in fisheries work. The major disadvantage of such equipment is its large size, which presents difficulties in both storage and transport. Detailed below is a folding version, made from wood and plastic mesh, which is designed to overcome these problems. Although measurements are given in terms of a general formula, for many purposes a cage 120 x 60 x 60 cm may be the most useful.

Lengths of planed softwood 20 x 50 mm are required to make the base, side, and end frames. In addition, two spacer pieces the same length as the cage (one 25 x 40 mm and another 25 x 65 mm), four attaché case clips, 10 brass butt hinges, a carrying handle, suitable plastic netting, screws, netting staples, two lengths of angle steel, and polyurethane varnish are needed for each cage.

The general design of the cage and construction details are shown in Fig. 1. Overall size is taken to be length(L) x width(W) x height(H). Five rectangular wooden frames are made up, using half-lap joints at the corners, to the following specifications:

Base	L x W
Two ends	(W – 50 mm) x (H – 25 mm)
First side	L x (H – 65 mm)
Second side	L x (H – 90 mm)

Before assembling the cage, staple the netting to the inside of these five frames. Then hinge the end units to the base so that they fold inward. (Note that, due to internal hinging of the ends, the maximum height of the end piece cannot be more than one-half of the length less 25 mm; i.e., the overall height of the cage cannot exceed one-half of its length). After fastening the spacer pieces to the base, the tallest side frame is hinged to the 40-mm spacer and the lowest side frame to the 65-mm spacer so that they also fold inward. Attaché caseclips are positioned to locate and fasten the top corners when the cage is open, and a carrying handle is screwed to the 65-mm spacer for use when the cage is folded. Angle steel is then screwed to the base edges to act as ballast against the natural bouyancy of wood and the whole structure is given a coating of polyurethane varnish.

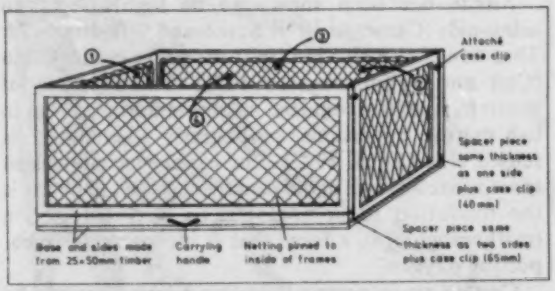
Attaché case clip
Spacer piece same thickness as one side plus case clip (40 mm)
Spacer piece same thickness as two sides plus case clip (65mm)
Base and sides made from 25×50mm timber
Carrying handle
Netting pinned to inside of frames

Fig. 1. A general view of the collapsible fish-holding cage. The netting-covered frames which are hinged to the base are folded inward in the order 1, 2, 3, 4.

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Accepted 20 January 1982

Effect of Acclimation Time on Nitrite Movement Across the Gill Epithelia of Rainbow Trout: The Role of "Chloride Cells"

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ABSTRACT: Plasma nitrite concentration and the number of lamellar chloride cells were directly correlated in rainbow trout (*Salmo gairdneri*) transferred from isotonic seawater to fresh water, during a 96-h acclimation period. After a 48-h acclimation, plasma nitrite levels of fish exposed to 175 mg/L of nitrite for 2.5 h were significantly higher than those of fish acclimated for 24 h. There was a 9.5-fold increase in the number of lamellar chloride cells during this period. These results suggest that nitrite may be transported across gill epithelia by means of the branchial anion exchange mechanism attributed to lamellar chloride cells in fresh water.

Nitrite has been shown to be highly toxic to salmonids (Cameron 1971; Smith and Williams 1974; Thurston et al. 1979: 158–162) and other cultured fish (Colt and Tchobanoglous 1976). The presence of nitrite is widely recognized as a potential problem in fish culture operations, particularly when water is reused (Liao et al. 1972). The exact mechanisms of toxicity are not well understood. One effect of nitrite is the oxidation of hemoglobin to ferrihemoglobin (methemoglobin), a form that is incapable of transporting oxygen.

Certain environmental ions have been reported to afford protection against the development of ferrihemoglobinemia and nitrite toxicity. Crawford and Allen (1977) found that the presence of environmental calcium ions in fresh water and seawater reduced nitrite toxicity in chinook salmon (*Oncorhynchus tshawytscha*). Wedemeyer and Yasutake (1978) found a similar protective effect of environmental calcium in steelhead trout (*Salmo gairdneri*). Perrone and Meade (1977) showed that the tolerance of coho salmon (*Oncorhynchus kisutch*) to nitrite increased with increasing chloride concentration. Similar results were obtained with steelhead trout (Wedemeyer and Yasutake 1978) and channel catfish (*Ictalurus punctatus*; Tomasso et al. 1979).

Colt and Tchobanoglous (1976) and Wedemeyer and Yasutake (1978) suggested that nitrite passes through the gill epithelium in the lipid-soluble form (Hunn and Allen 1974) of gaseous nitrous acid. Recent studies have indicated that nitrite may also be actively pumped across the gill epithelium (Bath and Eddy 1980; Meade and Perrone 1980; Tomasso et al. 1980). Bath and Eddy (1980) further demonstrated that rainbow trout (*Salmo gairdneri*) are able to concentrate nitrite in the blood. They suggested that the gills have the ability to transport nitrite against a concentration gradient by means of the branchial anion exchange mechanism.

The gill epithelia of fish have been separated into two main functional types. The primary epithelium of the filament contains the seawater chloride cells and functions in the excretion of ions. The secondary epithelium, which covers the secondary lamellae, contains the freshwater chloride cells. These cells are believed to function in the inward transport of ions (Laurent and Dunel 1980). The present study was undertaken to determine the effect of increasing the number of lamellar chloride cells on nitrite uptake in rainbow trout.

Materials and Methods

Yearling rainbow trout (average weight, 194 ± 10 g; average length, 27 ± 4 cm) were obtained from Seafarms, Inc., Old Lyme, Connecticut. One group of fish was acclimated to seawater that was approximately isotonic (salinity about one-third full strength; Na^+, 130 meq/L; K^+, 3.3 meq/L; Cl^-, 160 meq/L; Ca^{++}, 140 mg/L) prepared with Instant Ocean salts. These fish were maintained in 189-L (50-gal) glass aquaria with under-gravel filtration for at least 2 weeks before freshwater acclimation and exposure to nitrite. A freshwater group was maintained in 1,136-L (300-gal)

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circular tanks in a water re-use system at the University of Rhode Island aquaculture facility. Nitrite levels in this system were < 1 mg/L at all times.

Each experimental group was allowed to acclimate to fresh water (Cl^-, 25 mg/L; Ca^{++}, 18 mg/L) in 114-L (30-gal) glass aquaria with under-gravel filtration for a specified length of time ranging from 0.5 to 96 h before exposure to nitrite. Fish were not fed during the acclimation period or for 24 h before the 0.5-h acclimation experiments. Water temperature was a constant 14 ±1°C and the pH was 6.3 during the acclimation periods and the experiments. Dissolved oxygen levels were kept at 7.5–8.5 mg/L with aeration. Nitrite levels in aquaria were < 1 mg/L throughout the acclimation period.

Following acclimation to fresh water, static exposures were conducted in 114-L aquaria. The concentration of nitrite was brought to 175 mg/L by using sodium nitrite and the exposure period lasted 2.5 h. No mortalities occurred during exposure to nitrite. At the end of the exposure period fish were sacrificed by a blow to the head. Blood samples were drawn from the caudal vessels in heparinized syringes and analyzed for plasma nitrite and ferrihemoglobin. A Fisher Accumet digital ion meter with an Orion nitrogen oxide electrode was used to measure plasma nitrite. The minimum limit of detection for this technique was 6 mg/L. Percent ferrihemoglobin was determined by using the method of Dubowski (1960) and a buffer of pH 7.4.

Gill tissue was prepared as described by Copeland (1948). The third gill arch on the left side was removed immediately after death and fixed in Regaud's fixative. Routine histological techniques were used. Sections were cut at 6 μm and stained by Altmann's acid fuchsin method for mitochondria. With this method the chloride cells appeared as eosinophilic, granular cells and were easily differentiated from other cell types of the gill by an abundance of mitochondria. A number of different sections were cut and stained for each gill in an attempt to get similar cuts for each fish.

The total number of filament and lamellar chloride cells were counted in two separate areas for each fish. The number reported is the average of the two areas. The area was defined by counting the cells contained on fifty lamellae (25 on each side) and the accompanying filament. The first 25 lamellae from the base toward the tip of the filament were always counted. Laurent and Dunel's (1980) classification of filament and lamellar chloride cells was followed. A typical section (base of filament not shown) containing 14 (7 on each side) lamellae is shown in Fig. 1. Typical filament and lamellar cells are illustrated in Fig. 2. Since all lamellae were not the same length, this method gives only a rough quantification.

Statistical procedures included standard error of the mean (recorded as mean plus or minus one standard error) and Student's *t*-test for significant differences as described by Lentner (1972). The level of significance is accepted as being $P \leq 0.05$ (unless otherwise indicated).

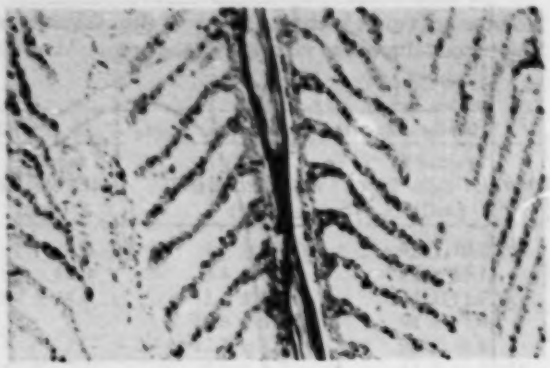

Fig. 1. Photomicrograph of a typical section of a freshwater gill. Lamellar chloride cells and filament chloride cells are apparent. (160X)

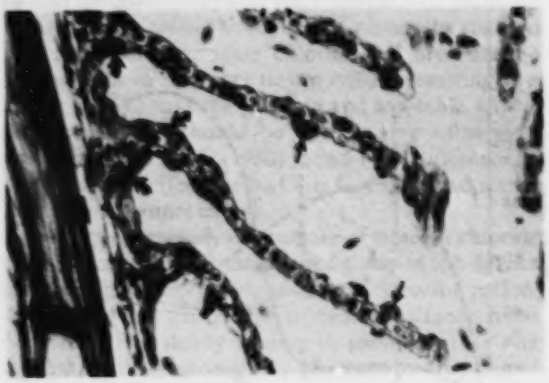

Fig. 2. Photomicrograph at a higher magnification, illustrating lamellar (straight arrow) and filament (curved arrow) chloride cells. (400X)

Results

Fish transferred from isotonic seawater to fresh water showed a significant ($P \leq 0.001$) increase in plasma nitrite concentration after a 48-h acclimation and 2.5-h exposure to nitrite, as compared with either 0.5- or 24-h acclimation (Fig. 3). The number of lamellar chloride cells increased 9.5-fold after 48 h of acclimation and there was a simultaneous rise in ferrihemoglobin levels (Table 1). Plasma nitrite concentrations and the number of lamellar chloride cells

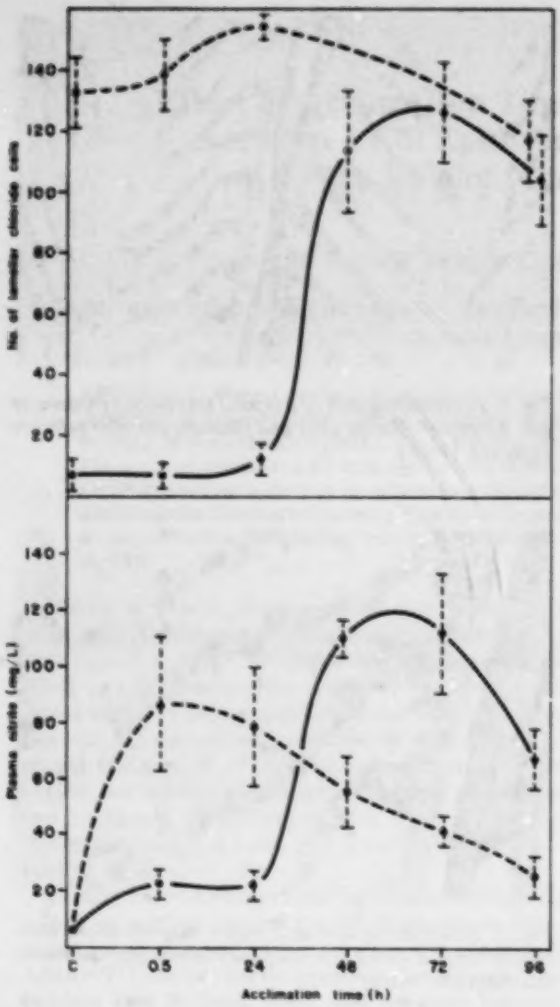

Fig. 3. Plasma nitrite and number of lamellar chloride cells for control rainbow trout (C) maintained in fresh water (- - -) and isotonic seawater (—), followed by transfer to freshwater acclimation tanks and 2.5-h exposure to 175 mg/L nitrite. All control plasma nitrite levels were < 6 mg/L (lower limit of detection for technique employed).

remained high after 72- and 96-h acclimation followed by 2.5-h exposure to nitrite. The number of filament chloride cells remained constant throughout the acclimation period (Table 1).

In the group of fish maintained in fresh water and exposed to nitrite, the number of chloride cells on the lamellae (Fig. 3) and the filament (Table 1) changed little during residence time in aquaria and exposure to nitrite. There was a steady decline in plasma nitrite concentration, although only the data for 96-h acclimation and exposure to nitrite were significantly different from those for the 0.5-h acclimated group.

Control fish in fresh water and acclimated to one-third seawater that were not exposed to nitrite had plasma nitrite levels beneath the limit of detection for the technique employed (Fig. 3) and ferrihemoglobin levels of 1.2 ± 0.2%. In the freshwater control group the number of lamellar chloride cells was similar to the values reported for the freshwater-maintained, nitrite-exposed groups.

In the control group acclimated to one-third seawater, the number of lamellar chloride cells was similar to values reported for the corresponding nitrite-exposed group after 0.5- and 24-h acclimation in fresh water (Fig. 3).

Discussion

Several authors have suggested that nitrite may diffuse across the gill epithelium as a lipid-soluble gas, nitrous acid (HNO_2), based on the observed effects of environmental pH, calcium ions, and pKa on nitrite toxicity (Colt and Tchobanoglous 1976; Wedemeyer and Yasutake 1978). However, the protective effect of environmental chloride ions against nitrite toxicity (Perrone and Meade 1977; Tomasso et al. 1979) and recent work by Tomasso et al. (1980) support the hypothesis that transepithelial nitrite movement is mediated primarily by means of an electrogenic anion "pump" which is normally responsible for active chloride uptake from hypotonic media.

Results of the present study show an increase in the rate of plasma nitrite accumulation with acclimation of rainbow trout from an isotonic to a hypotonic environment. Fish survival after transfer from seawater to fresh water requires a rapid reduction of branchial ionic permeability (Evans 1975). This reduced permeability must be maintained, even after active NaCl uptake has been initiated (Motais and Garcia-Romeu 1972). It is therefore unlikely that high plasma nitrite levels after 48 h are due to increased membrane permeability.

These results further indicate that rainbow trout respond to a hypotonic environment by producing lamellar chloride cells. Conditions that would tend to exaggerate ion losses (such as skin wounds, external fungal infections, or the transfer of fish from fresh to deionized water) have been shown to stimulate proliferation of these cells (Laurent and Dunel 1980). In addition, Girard and Payan (1980) have demonstrated that in freshwater trout, $^{24}Na^+$ and $^{36}Cl^-$ enter at the gill only through the secondary lamellae.

Bornancin et al. (1980), who studied the anion-dependent ATPase found in the gills of freshwater rainbow trout, confirmed its participation in the exchange of Cl^- and HCO_3^-. They also found the exchange could be stimulated by other anions, such as

Table 1. Percent ferrihemoglobin and number of filament chloride cells ($\bar{x} \pm$ one standard error) for rainbow trout maintained in fresh water and isotonic seawater, followed by transfer to freshwater acclimation tanks and 2.5-h exposure to 175 mg/L nitrite.

No. of fish/tank	Time in freshwater tanks (h)	Freshwater-maintained		Isotonic seawater-maintained	
		Ferrihemoglobin (%)	Filament chloride cells (no.)	Ferrihemoglobin (%)	Filament chloride cells (no.)
5	Controls	1.2 ± 0.2	97 ± 8.0	1.2 ± 0.2	92 ± 10.4
5	0.5	12.6 ± 3.0	93 ± 11.0	3.4 ± 0.5	82 ± 15.1
5	24	13.8 ± 3.3	104 ± 14.4	3.4 ± 0.3	94 ± 11.7
4	48	8.6 ± 1.9	-	14.6 ± 1.6	98 ± 7.4
4	72	6.5 ± 0.7	-	12.7 ± 1.6	98 ± 5.8
5	96	6.5 ± 1.2	88 ± 7.1	9.6 ± 1.7	87 ± 12.2

sulfate. If lamellar chloride cells are involved in active chloride uptake in fresh water, perhaps similar anions, such as nitrite, use the same pathway for branchial entry into the blood. The direct correlation between plasma nitrite values and numbers of lamellar chloride cells (Fig. 3) would tend to support this hypothesis.

Crawford and Allen (1977) and Wedemeyer and Yasutake (1978) reported that the toxicity of nitrite is significantly reduced in the presence of environmental calcium ions. The present study has implicated lamellar chloride cells in the branchial movement of nitrite across fish gills in fresh water. It remains unclear whether the proliferation of chloride cells in these experiments was in response to reduced environmental chloride alone or in combination with reduced calcium. Laurent and Dunel (1980) believed that chloride cell proliferation, after transfer of fish from fresh to deionized water, was in response to increased ion flux stimulated by calcium removal. Potts and Flemming (1971) found that removal of calcium increased the sodium permeability of the euryhaline teleost, *Fundulus kansae*, in both fresh water and seawater, and Bornancin et al. (1972) showed a doubling of the sodium influx rate in seawater-acclimated eels (*Anguilla anguilla*) with calcium removal. The stenohaline marine pinfish (*Lagodon rhomboides*) can survive indefinitely in fresh water if sufficient calcium ions are present (Carrier and Evans 1976). In fresh water, environmental calcium serves to reduce branchial ion efflux sufficiently so that internal NaCl levels remain within physiological limits, through the initiation of compensatory ion uptake mechanisms.

The information discussed above, taken with the results of the present study, suggest a possible explanation for the findings of Crawford and Allen (1977) and Wedemeyer and Yasutake (1978). Assuming the competitive Cl^-:NO_2^- uptake hypothesis proposed by Perrone and Meade (1977) to be correct, high levels of environmental calcium would tend to reduce the branchial efflux of chloride in hypotonic environments, so that the chloride uptake requirement, and therefore nitrite uptake, would be reduced. Conversely, reduced calcium would stimulate chloride cell proliferation and activity in response to ion efflux, resulting in a higher uptake rate for chloride and available nitrite. This hypothesis should be tested using salmonids, since Tomasso et al. (1980) found little difference in the protective effects of NaCl or $CaCl_2$ against nitrite toxicity in channel catfish.

In the present study the number of lamellar chloride cells in the freshwater-maintained group of fish did not change appreciably throughout the acclimation period, suggesting that no change in osmoregulatory stress occurred. The steady decline in plasma nitrite and percent ferrihemoglobin may have been due to reduced physical activity and the absence of feeding with transfer to a static system. These fish entered a resting metabolic state with a concurrent reduction in both oxygen consumption and gill respiratory surface area (Rao 1968; Steen and Kruysse 1964). Thus both chloride efflux and the number of active chloride cells could have been reduced, resulting in a lower uptake requirement for chloride and subsequently reducing nitrite uptake.

Acknowledgments

The authors thank R. E. Wolke, James Bubien, Virginia Reed, and Nannette Anctil for their assistance in the preparation of this paper, and the technical staff of the Marine Pathology Laboratory of the University of Rhode Island for histological preparation of gill tissues. This research was jointly supported by the Rhode Island Agricultural Experiment Station (Contribution No. 2004), University of Rhode Island, Kingston; the U.S. Fish and Wildlife Service, Contract No. 14-16-0008-2135; and the NOAA-National Sea Grant Program, NA-79AA-D-00096.

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Accepted 21 January 1982

Performance of Rainbow Trout Fed Gelatin-Bound Diets of Fish Protein Concentrate of Casein Containing 25 to 45 Percent Herring Oil[1]

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ABSTRACT: Five diets based on fish protein concentrate-gelatin (FPC), ranging in herring oil content from 25 to 45%, and one casein-gelatin based diet containing 35% herring oil were fed to rainbow trout (*Salmo gairdneri*) for 95 days to evaluate nutrient assimilation of diets with high caloric densities. Digestible energy densities of diets ranged from 4.36 to 6.19 kcal/g. Diets were formulated to be isonitrogenous (38% crude protein). Alpha-cellulose, which is considered to be indigestible by fish, was the only carbohydrate used in formulation of diets. Weight gains were higher ($P < 0.01$) for fish fed the FPC than for fish fed the casein-gelatin diet. Oil level in the diet was highly related ($P < 0.05$) to feed conversion efficiency (FC; weight of feed consumed/live weight gain), having a correlation coefficient of −0.88. The FPC diet containing 35% herring oil had a higher FC (0.821, $P < 0.05$) than the casein diet with 35% herring oil (FC, 0.971). All FPC diets had a higher dietary energy conversion rates than the casein-gelatin diet ($P < 0.01$), which indicated that casein is limiting with respect to protein quality for trout. A trend for improving protein conversion was observed as the caloric density of the diet increased. The FPC diets were more efficient ($P < 0.05$) in supporting conversion of dietary protein into tissue than was the casein diet. The overall desirability of the fish as determined by an organoleptic taste panel was lower ($P < 0.05$) for fish fed diets with high levels of herring oil, but fell within acceptable limits.

The science of fish nutrition has not developed to a level of knowledge equivalent to that of the other domestically important livestock species. Optimum levels of nutrients and nutrient interactions are not well defined.

Semipurified diets for trout were initially formulated (McLaren et al. 1947) by using the requirements known for rats. Improvements have since been made in the development of semipurified diets (Halver et al. 1964; Shanks et al. 1962; Lee et al. 1967; Castell et al. 1972; Yu and Sinnhuber 1972; Phillips et al. 1946, 1947) that enabled researchers to evaluate the use of nutrients by trout.

The natural diets of trout are primarily composed of proteins and lipids. Carbohydrates are commonly included in commercial trout diets, as an inexpensive energy source, but Phillips and Brockway (1959) found that complex carbohydrates, such as starch, are not used effectively. Bergot (1979) found that diets containing glucose produced higher average daily gains than diets containing starch. Decreased dietary energy usage was reported when carbohydrates were fed to rainbow trout (*Salmo gairdneri*; Austreng et al. 1977). The enzymes involved in carbohydrate metabolism in trout have been found to be similar to those in diabetic mammals (Nagayama et al. 1972, 1973). In carp (*Cyprinus carpio*), the metabolism of protein and lipids was found to be twice as rapid as the rate observed for metabolism of carbohydrates by Nagai and Ikeda (1973). Abnormal changes, such as high levels of glycogen in the livers, have been observed when diets high in carbohydrate were fed to salmonids (Phillips and Brockway 1959).

The role of carbohydrates in salmonid nutrition is an area of conjecture at the present time. It is generally accepted that carbohydrates are not essential for normal growth and serve only as a source of energy which could be provided by either lipid or protein.

Pieper and Pfeffer (1980) showed that carbohydrates were used as efficiently as soybean oil by trout, but noted enlarged livers and accumulation of fat in the liver. The growth rate of trout was found to be depressed when carbohydrates were increased in trout diets (Edwards et al. 1977).

Fish diets containing as much as 24% salmon oil produced good growth and feed conversion (Lee and Putnam 1973; Reinitz et al. 1978). The use of lipid levels greater than 25% in trout diets has not been investigated, since they increase deposition of body fat, which is thought to result in an inferior product.

[1] Technical Paper 5930, Oregon Agriculture Experiment Station. This work is a result of research sponsored (in part) by the Oregon State University Sea Grant College Program supported by NOAA Office of Sea Grant, U.S. Department of Commerce, under Grant No. NA-79AA-D-00106.

The objective of the present research was to evaluate the assimilation of diets with high caloric densities (based on herring oil) by rainbow trout. The effect on growth rate, feed conversion, protein conversion, energy conversion, and the acceptability of the edible tissue were evaluated.

Methods

Six semipurified diets containing varying levels (25 to 45%) of feed-grade herring oil were evaluated. Fish protein concentrate (FPC) was used as the supplemental protein base for five of the diets and vitamin-free casein for one. Gelatin was incorporated at 7.8% in all diets as a binding aid. Composition of the diets is given in Table 1. All six diets were formulated to contain 38% crude protein (CP); the only dietary variables were alpha-cellulose and herring oil. Since alpha-cellulose is considered to be indigestible by fish, diets were essentially carbohydrate-free, which would simulate closely the fish's natural diet.

Fingerling rainbow trout of the Mt. Shasta strain (mean initial weight, 4.9 g) were the test animals. Each diet was fed to three groups of 50 fingerlings each. Groups were placed in 21-L perforated plastic buckets (designed for the rearing of small lots of fingerlings) for the first 4 weeks; fingerlings were then transferred to 42-L tubs for the rest of the trial. Oxygen concentration of the water was maintained at 10 mg/L by jetting water into each tub. Well water with a constant temperature of 11.5°C was used.

Fish were fed for 5 consecutive days and then fasted for 2 days. During each feeding period of 8 h, fish were fed 5 to 6 times, and this regime was continued for the entire trial. Fish were fed as long as they were actively feeding and daily consumption was recorded.

Diets were prepared every 2 weeks from pre-mixes that had been made up before the start of the trial. A vitamin pre-mix, herring oil, and a measured volume of hot water (60°C) were added to each preweighed pre-mix. After mixing, diets were refrigerated overnight to allow the gelatin to set. The semisolid diets were then cut into cubes of the appropriate size, preweighed into the approximate amounts required for 1 day's feeding period, and frozen.

Groups of fish were individually weighed every 2 weeks and counted to determine mortality. Fish were weighed after being fasted for a period of 2 days to minimize the effect of ingested food.

Proximate composition (CP, ether extract, moisture, and ash) of duplicate groups was determined for each of the six treatments, and a composite sample from the initial population was analyzed by using the methods given in the Association of Official Agricultural Chemists (Horwitz 1975). Feed and energy and protein converison coefficients were computed on the basis of the analytical results.

Table 1. Composition of test diets.

Ingredients	Fish protein concentrate[a]					Casein[a]
	25	30	35	40	45	35
Fish protein concentrate	29.4	29.4	29.4	29.4	29.4	
Casein						29.6
Gelatin	7.8	7.8	7.8	7.8	7.8	7.8
Herring oil	25	30	35	40	45	35
Alpha-cellulose	31.8	26.8	21.8	16.8	11.8	21.6
Carboxymethyl cellulose	1.2	1.2	1.2	1.2	1.2	1.2
Alpha-tocopherol[b]	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.7	0.7	0.7	0.7	0.7	0.7
Mineralsupplement[c]	1.9	1.9	1.9	1.9	1.9	1.9
Vitaminsupplement[d]	1.9	1.9	1.9	1.9	1.9	1.9

[a] Percent herring oil added to diet, expressed on a dry-matter basis.
[b] IU of vitamin E per gram of diet.
[c] Mineral supplement provided (milligrams per kilogram of diet): calcium carbonate ($CaCO_3$), 0.4; calcium phosphate ($CaHPO_4 \cdot 2H_2O$), 14; potassium phosphate (K_2HPO_4), 1.5; potasium sulfate (K_2SO_4), 1.3; sodium chloride (NaCl), 0.58; sodium phosphate ($NaHPO_4 \cdot 6H_2O$), 0.4; magnesium oxide (MgO), 0.48; ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$), 0.11; manganese carbonate ($MnCO_3$), 0.08; cupric carbonate ($2CuCO_3Cu(OH)_2$), 0.006; zinc carbonate ($ZnCO_3$), 0.015; potassium iodide (KI), 0.0001; sodium fluoride (NaF), 0.0003; cobalt chloride ($CoCl_2$), 0.0038; and citric acid ($C_6H_8O_7 \cdot H_2O$), 0.13.
[d] Vitamin supplement provided (milligrams per kilogram of diet, except as otherwise noted): thiamin, 64; riboflavin, 144; niacinamide, 512; biotin, 1.6; calcium-pantothenate, 288; pyridoxine, 48; folic acid, 1,200; and para-amino-benzoic acid, 400; 400 IU/kg vitamin D_3; 25,000 IU/kg vitamin A.

Gross energy consumption was computed for the diets, using 5.6 kcal/g for protein and 9.3 kcal/g for herring oil; these values are summarized in Table 2.

Condition factors (K-value) were computed to determine if changes in physical condition were caused by feeding high caloric density diets. To calculate the K-value we used the equation K = weight in grams ÷ (length in cm)^3 x 100.

After completion of the 95-day trial, composite groups of fish were formed for the respective treatments and continued on the test diets until they weighed about 200 g each. The fish were then sacrificed and the edible tissues subjected to an organoleptic evaluation by 10 trained panelists to determine their texture, flavor, and overall acceptability.

Results and Discussion

The average initial weight was 247 ±19 g for all groups (50 fish per group), and average final weight was 1,354 ± 316 g. Average individual fish weight increased from 4.9 to 27.1 g during the 95-day trial. Average daily gains for each treatment are presented in Table 2. Weight gains for groups fed the FPC diet

Table 2. Performance of rainbow trout fed diets of high caloric density[a].

Diet[b]	Average daily gain (g/day)	Feed conversion[c]	Energy kcal/g of gain	Energy conversion (%)	Protein conversion	Condition factor
Fish protein concentrate						
25	0.251[w]	0.891[w]	3.35[w]	58.6[w]	61.3[w]	1.13
30	0.242[w]	0.882[w]	3.76[w,x]	54.3[w]	62.2[w]	1.16
35	0.240[w]	0.821[x]	3.82[w,x]	56.0[w]	63.0[w]	1.18
40	0.226[w]	0.791[x]	4.14[x]	54.3[w]	64.1[w]	1.16
45	0.259[z]	0.756[y]	4.20[x]	55.3[w]	66.3[w]	1.19
Casein						
35	0.181[x]	0.971[z]	4.53[y]	41.3[x]	50.4[x]	1.21

[a] Means in the same column followed by different letters are different ($P < 0.05$).
[b] Percent of herring oil in diet, expressed on a dry-matter basis.
[c] Feed consumed divided by live weight gain.

were not different ($P > 0.05$) for the different levels of herring oil, but were higher ($P < 0.05$) than gains in the groups fed the casein-gelatin diet.

Feed conversion (FC) coefficients (dry weight of feed/live weight gain) for the six diets tested are summarized in Table 2. As the herring oil content in the FPC-based diets was increased, FC improved ($P < 0.05$). More efficient feed conversion was achieved with all the FPC diets than with the casein-gelatin based diet ($P < 0.05$). The efficiency of FC was improved for all diets as the length of time on the diet increased (correlation coefficient, 0.900). The FPC diet with 35% herring oil had an FC of 0.821, compared with 0.971 for the casein diet with 35% herring oil.

Dietary energy required per unit of gain increased as the herring oil content was increased. All the FPC diets required less energy per unit of gain than did the casein diet ($P < 0.05$).

The proximate composition of fish from the six treatments is shown in Table 3. When differences in caloric content of the tissue were adjusted, energy conversion was approximately equal for the FPC diets, as shown in Table 2. FPC diets were more efficient than the casein diet ($P > 0.05$) in providing dietary energy.

Protein conversion coefficients for the various diets are summarized in Table 2. There was a nonsignificant ($P > 0.05$) trend toward improved protein conversion as the caloric density of the diet increased. The FPC-based diets were more efficient than the casein-based diet in converting dietary protein into tissue ($P < 0.05$).

The K-value for a normal fish is about 1, with values less than 1 tending towards leanness and values greater than 1 tending toward obesity. Computed values for the test fish are summarized in Table 2. All groups were considered to be within the normal range, but on the heavy side.

Table 3. Proximate percentage composition of fish.[a]

Diet[b]	Crude protein	Ether extract	Moisture	Ash
Fish protein concentrate				
25	16.8	13.9	68.9	2.16
30	16.3	15.4	68.3	2.10
35	16.1	16.2	67.4	2.07
40	16.1	17.4	66.1	2.07
45	15.6	18.5	65.5	2.03
Casein				
35	15.6	14.1	68.5	2.19
Initial[c]	16.6	5.9	76.8	2.21

[a] Analysis of ten fish from each treatment group at the termination of the experiment.
[b] Percent of herring oil in diet expressed on a dry-matter basis.
[c] Sample from initial population.

The organoleptic panel evaluation of the edible tissues is summarized in Table 4. The overall desirability (texture and flavor) of the fish was approximately the same, except for fish fed the FPC-40% herring oil diet, which were found to be less acceptable ($P < 0.05$). Evaluation of the flavor varied, but tended to decrease as the amount of herring oil in the diets increased.

Results of the present study indicate that rainbow trout are able to use diets of very high caloric density, in which marine lipids are the primary energy source, with a relatively high degree of efficiency. Trout were able to convert about 56% of the dietary calories consumed into body tissues in the FPC-based diets and 41% in the casein-gelatin based diet. Protein and

Table 4. Results of taste panel evaluation.[a,b]

Diet[c]	Flavor	Texture	Desirability
Fish protein concentrate			
25	6.36[w,y,z]	6.31[w]	6.31[w]
30	6.50[w,z]	6.39[w]	6.36[w]
35	5.89[w,x,y]	6.47[w]	6.03[w]
40	5.42[w,y]	5.78[w]	5.44[x]
45	5.81[y]	6.31[w]	5.97[w,x]
Casein			
35	6.56[z]	6.47[w]	6.36[w]
LSD[d]	0.61	0.51	0.57

[a] Scores ranged from 1 to 10, with 10 being the highest, and 1 the lowest.
[b] Means in column followed by different letters are different ($P < 0.05$).
[c] Level of herring oil in diets expressed on a dry-matter basis.
[d] Least significant difference ($P < 0.05$).

feed conversion improved as the caloric density increased in the FPC-based diets; the FPC-35% herring oil diet was more efficient than the casein-35% herring oil diet. That the FPC-based diets produced superior responses in all the performance variables evaluated suggests that the protein quality of FPC exceeds that of casein for rainbow trout.

Results indicate that trout can efficiently use semi-purified diets of high caloric density composed of protein and lipids in the production of body tissues.

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Accepted 3 February 1982

"Green Water" Culture of Grass Shrimp in Saltwater Aquaria

Grass shrimp (*Palaemonetes pugio*) occur in brackish water (salinity, 1–15°/oo) along the Gulf and Atlantic Coasts, where they are used for fish food, bait, and toxicological testing (Huner 1979; Tyler-Schroeder 1979). Grass shrimp have been successfully reared in ponds, flow-through aquaria, and finger bowls where water quality was maintained by frequent water changes (Chew 1981; Little 1968; Tyler-Schroeder 1978, 1979). These methods are either too expensive (pond construction) or labor intensive. "Green water" (the addition of algae to the culture water) larviculture has been used to rear *Macrobrachium rosenbergii* and fishes (Cohen et al. 1976; Fujimura 1966; Houde 1975, 1977, 1978; Wickins 1972). A laboratory method using green water was developed to rear juvenile grass shrimp of uniform size in salt-water aquaria for toxicological testing at the Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

Equal amounts of the wild and cultured algae were combined to make up the 11.4 L (3 gal) of algae that were added to each shrimp culture tank. Cultures of wild algae, predominantly *Chlorella*, were reared in two outdoor 37.9-L (10-gal) aquaria containing water at a salinity of 15 °/oo (Instant Ocean). *Chlorella* was maintained in pure culture for the laboratory rearing of amphipods and copepods.

Between May and September 1980, 57 gravid grass shrimp were collected with a dip net from Davis Bayou, Ocean Springs, Mississippi. The chelipeds were clipped, and the female shrimp were placed in the aquaria in rectangular plexiglass spawning chambers with 3.3-mm (0.13-in.) netting on the bottom that had been designed for sheepshead minnow (*Cyprinodon variegatus*) spawning (A. R. Lawler, Gulf Coast Research Laboratory, personal communication).

Grass shrimp were spawned and reared in two 110-L (29-gal) aquaria. The culture medium consisted of Instant Ocean adjusted to a salinity of 15 °/oo with aged tap water. The algae that were added to each culture tank resulted in a visibility of 3 cm. The 25°C (77°F) temperature was maintained by an aquarium heater and oxygen saturation by means of an airstone and pump. Constant illumination was provided by two fluorescent bulbs placed against the rear of each aquarium. Adult and larval grass shrimp were fed brine shrimp (*Artemia*) and Tetramin daily at 0800 h. Brine shrimp cysts and waste were netted off the bottom of the aquaria weekly, and tap water was added as needed to maintain water levels. The culture medium was not changed in the rearing tanks and algae were not added to the tanks after the initial seeding.

Two groups of gravid grass shrimp were successfully spawned in each of the two aquaria at various times between May and September 1980. The four groups contained 6, 5, 28, and 13 females, and the percentages of successful spawning were 66.7, 100.0, 85.7, and 76.5, respectively; overall spawning success was 82.1%. More than 5,000 larvae were hatched from 46 gravid females. The four broods were spawned and reared in the same culture water for more than a year without the water being changed or filtered. Cohen et al. (1976) reported that algae in larviculture purified the medium primarily by assimilating ammonia, but that the success of this process depended on a precarious balance between the uptake of ammonia by the algae and the production of ammonia by the grazing *Artemia* and the larvae. Fujimura (1966) suggested that constant water exchange and the addition of algae would be necessary for successful larviculture. In the present study the ecological balance was easily maintained by the weekly removal of wastes and sediments, with neither exchange of water nor addition of algae. Success of this method could have been due to the dense algal bloom; although the algal cell concentration was not determined during the present study, it was much greater than that in the rearing facilities used by Houde (1975), based on my observation of his rearing facilities. Cohen et al. (1976) used an algal concentration of 5×10^6 cells/mL of *Tetraselmis* and 2×10^7 cells/mL of *Phaeodactylum* for the larviculture of *Macrobrachium*. The grass shrimp culture medium used in the present study was several times more dense than this.

Three 18.9-L (5-gal) rearing tanks were set up as above for a feeding study and 25 larvae were placed in each aquarium. Diets of Master Mix Starter 970, Tetramin, or brine shrimp were fed to the larvae in these aquaria to determine which foods could be used for grass shrimp larviculture. The feeding study was conducted for 103 days. The shrimp fed on brine shrimp had the lowest mortality (16%); mortality of shrimp fed Tetramin (44%) was less than for those fed Master Mix (52%). Growth of shrimp fed the brine shrimp diet (19.24 mm TL) was not significantly different from that of shrimp fed the Master Mix diet (20.00 mm TL), but the growth of shrimp fed the Tetramin diet (16.86 mm TL) was significantly less

than that of shrimp on the other diets. Although no definite conclusion can be drawn from these feeding trials, larval grass shrimp were successfully reared on diets of brine shrimp, Master Mix, or Tetramin. Thus, if the cost of brine shrimp eggs is prohibitive, shrimp larvae can be reared exclusively on dry foods.

The green water method used at Gulf Coast Research Laboratory for rearing grass shrimp was simple, inexpensive, and easy to maintain. Use of this method eliminated the need for ponds, flow-through aquaria, or finger bowls, and the method was not labor intensive.

Acknowledgments

I thank Adrian R. Lawler, Steve Shepard, and Patrick McKinney for their support and assistance. This study was funded by the Toxicology Program of the Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

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Accepted 13 January 1982

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Observations of a 4° C Groundwater Source as a Release Site for Atlantic Salmon

Using a groundwater source in Iceland of exactly 4° C at all times, I conducted tests to determine whether (1) Atlantic salmon (*Salmo salar*) would smolt at this temperature, and (2) adult salmon would return to a smolt-release site of this temperature. Although answers to these questions are unlikely to be of practical significance, they are of physiological interest. The natural conditions described here not only are rare, but may also be considered basically inferior to those of release sites where water sources warm up in spring or early summer.

On 7 May 1966, 30 pre-smolt fingerlings were placed in a small wire basket with an opaque cover in an upwelling pool of a groundwater source which has direct access to the ocean and maintains a constant temperature of 4° C. The fish had been reared for

about 13 months in geothermal hatchery water at 10–12° C. The smolts in the basket received no feed. After 3 weeks in the basket no visible signs of smoltification could be detected, but at 4 weeks such signs became clear; after 6 weeks the smolts had bright silvery sides and had become restless, and their snouts were eroded by efforts to escape the basket. They were apparently in good physical condition, despite 6 weeks of starvation. At this stage, 27 smolts were released, and 3 were returned to their hatchery of origin for comparison with fish of the same age. The fish which had remained in 10–12° C hatchery water had not developed any signs of smoltification, in sharp contrast to those that had been held for 6 weeks at 4° C.

In spring 1980, 120 smolts were kept in a nylon-net cage in the test pool for 56 days and then released to the ocean. They fed intermittently during this period, but such feeding was probably of little significance. The smolts appeared in excellent condition when they were released from the cage directly to ocean water.

To evaluate the contention that Atlantic salmon do not enter a water source on return from the ocean unless some salmon fry or fingerlings remain in that water source (Nordeng 1971), in mid-June 1981 about 50 smolts were placed in the same cage that had been used previously, kept there for 84 days, and then released. A funnel was positioned at the entrance to the pool so that salmon could enter the pool, but not escape from it. On 6 September a female salmon that weighed 2.3 kg was caught in the test pool, resting in the shade of the nylon-net basket.

To these observations I wish to add that I am not aware of any test comparable to the one described in this note with Atlantic salmon in 4° C water. I doubt that such a test has been conducted before, simply because it would be impracticable, unless similar ecological conditions were available. Reference to smolting studies at varying and higher temperatures or involving photoperiodic effects would hardly be relevant in this context.

The reason fingerlings smolted in 4° C water and not in a hatchery water of constant 10–12° C is presumably the physiological requirement to "cool down" the fingerlings (to simulate winter effects) to induce smoltification. For the countries adjacent to the North Atlantic, such a need arises only in Iceland, where geothermal waters of constant temperatures of 10° C or more are used in salmon hatcheries. In Iceland in May and June, daylight lasts 24 h, thus excluding photoperiodic effects.

There are no spawning areas for salmon or trout near the mentioned test pool, and neither trout nor salmon ever stray or are caught anywhere near this area. There can be little doubt that the salmon that returned in 1981 was an adult developed from a smolt released from the pool in 1980. Even in the extremely unlikely event that this salmon was a "stray," it does not detract from the main lesson of this incident, namely that Atlantic salmon from the ocean may enter a freshwater source as cold as 4° C.

During a personal interview with Lauren R. Donaldson (Professor Emeritus, College of Fisheries, University of Washington, Seattle) in September 1981, he informed me that salmon smolts released from a water source of full oceanic salinity would return as adult fish to that same water source. He alluded to a general principle to the effect that adult salmon will return to the same "kind" of water from which they entered the ocean as smolts, whether the source is fresh or more or less saline water and whether it is cool or warm. My simple test in 4° C water appears to support this hypothesis.

In open rivers there are predictably very few, if any, instances where water temperature has not reached at least 6–8° C before Atlantic salmon smolts reared in these rivers are prepared to enter the ocean. Therefore in Iceland, as in other countries adjacent to the North Atlantic, experts refer to the fact that smolts do not leave the rivers to enter the ocean before the water temperatures have reached or exceeded this approximate range, and do not return to the rivers before they have warmed up appreciably in early summer. On these grounds, experts understandably might consider it unlikely that adult salmon would enter water as cold as 4° C.

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Accepted 19 January 1982

Influence of Temperature, Water Hardness, and Stocking Density on MS-222 Response in Three Species of Fish

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ABSTRACT: Three responses of rainbow trout (*Salmo gairdneri*), common carp (*Cyprinus carpio*), and fathead minnows (*Pimephales promelas*) to the anesthetic MS-222 (tricaine methanesulfonate) were identified: induction times to total loss of equilibrium decreased with increasing temperatures; resistance to MS-222 increased with increasing water hardness; and resistance to the drug increased when the density of test fish was increased from 0.3 to 0.9 g/L in rainbow trout, 0.4 to 1.2 g/L in carp, and 0.3 to 0.8 g/L in fathead minnows. Our results and others from the literature suggest that temperature, water hardness, and density of test fish, as well as pH and dissolved oxygen, may have significant interacting effects on the anesthetic properties of MS-222.

The anesthetic MS-222 (tricaine methanesulfonate) has received wide acceptance as being effective for fish and other cold-blooded animals (Schoettger 1967; Schoettger and Julin 1967; Wayson et al. 1976; Downes and Gerber 1974). It is the only fish anesthetic registered for fish by the U.S. Environmental Protection Agency and Food and Drug Administration (Meyer et al. 1976). Because of its relatively high cost, however, it would be advantageous to develop procedures that would reduce the amount needed to produce a given level of anesthesia in a short time. Toxicities of the drug to selected species of warmwater and coldwater fishes were determined by Marking (1967). Response levels to MS-222 were investigated in salmonids by Schoettger and Julin (1967) and in striped mullet (*Mugil cephalus*) by Sylvester (1975). Effective concentrations used in fish culture, fishery management, and research differ widely, and depend on such factors as size, maturity, and species (Bove 1962; Huish 1972).

Although the physiological effects of MS-222 anesthesia have been documented (Wedemeyer 1970; Houston et al. 1971; Houston and Woods 1972; Allen et al. 1972; Soivio et al. 1977; Strange and Schreck 1978), little attention has been directed toward environmental factors that might influence fish responses to MS-222. We therefore investigated the effects of water temperature, water hardness, and fish density on response times in rainbow trout (*Salmo gairdneri*), common carp (*Cyprinus carpio*), and fathead minnows (*Pimephales promelas*).

Materials and Methods

Fish used in these tests were obtained from hatcheries and acclimated for 7 to 10 days in the laboratory according to standard bioassay procedures (American Public Health Association 1971). Ranges in total lengths (mm) and average weights (g) were as follows: rainbow trout, 39–51 and 0.9; carp, 37–46 and 1.2; and fathead minnows, 38–45 and 0.8. Ten fish each were used for the water hardness and temperature tests, and

Table 1. Quantities of salts and characteristics of reconstituted waters at the National Fishery Research Laboratory, La Crosse, Wisconsin (Marking and Dawson 1973).

Water type	Salts added (mg/L)				pH range	Total hardness[a]	Total alkalinity[a]
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl			
Very soft	12	7.5	7.5	0.5	6.4–6.8	10–13	10–13
Soft	48	30	30	2.0	7.2–7.6	40–48	30–35
Hard	192	120	120	8.0	7.6–8.0	160–180	110–120
Very hard	384	240	240	16.0	8.0–8.4	280–320	225–245

[a] As mg/L $CaCO_3$.

5, 10, and 15 for the fish density tests. Fish densities (g/L) for rainbow trout increased from 0.3 to 0.9, for carp from 0.4 to 1.2, and for fathead minnows from 0.3 to 0.8. Each test was performed twice. All tests were conducted in static bioassay 15-L test tanks held under constant illumination. Desired water quality characteristics were produced by adding mineral salts to deionize water as specified in Table 1. General behavioral responses to MS-222 were given by Schoettger and Julin (1967). Anesthetic induction to stage 2 (i.e., loss of reflex activity and medullary collapse) was used as a response indicator in these tests. The time in minutes at which 50% of the test fish completely lost equilibrium was used as the measure of the effects of temperature, water hardness, and fish density on the anesthetic action of 40 mg/L MS-222. Times to initial response (i.e., loss of equilibrium) and final response (stage 2 anesthesia) for all fish in each test group were also recorded. The mean difference between initial and final responses between test groups was designated as anesthetic induction time.

Temperature and Water Hardness

The effects of temperature on induction time were studied at 12, 17, and 22°C in soft water (Table 1). The conditions of water hardness, pH, and alkalinity that are designated as soft in Table 1 were taken as "normal" and other test waters were expressed in relation to these values as very soft, hard, and very hard.

Induction times in four levels of water hardness at 12°C were determined in reconstituted water designated as very soft, soft, hard and very hard.

Induction times as a function of stocking density were determined at 12°C in normal water. Anesthetic effects were determined for 5, 10, or 15 fish per 15-L test tank. Regression analyses and *t*-tests were used to determine the significance of response times to MS-222 at different levels of temperature, water hardness, and stocking density. The *t*-tests were used to determine the significance of the greatest differences in mean response time both within and between species. Regression analyses of the transformed response times were calculated to determine the significance of increasing temperature, water hardness, and stocking density.

Results

As temperatures increased from 12 to 17°C, mean induction times decreased from 4.5 to 2.3 min in rainbow trout, from 18.5 to 11.5 min in common carp, and 7.3 to 2.4 min in fathead minnows. At 17 and 22°C, induction times for trout and fathead minnows ranged from 2.4 to 2.9 min. This range was significantly different from that for carp, which was 9.5 to 11.5 min (Table 2).

Table 2. Effects of temperature on time to loss of equilibrium in 50% of the test fish at 40 mg/L MS-222. Mean induction times at all three temperatures for rainbow trout and fathead minnows are significantly different from induction times for common carp ($P < 0.01$).

Species and temp (°C)	Induction time (min)		
	Mean	Range	SD
Rainbow trout			
12	4.5	3.0– 6.0	0.80
17	2.9	0.5– 5.0	0.12
22	2.3	0.5– 4.0	0.94
Common carp			
12	18.5	12.0–36.0	6.43
17	9.5	3.0–14.0	2.94
22	11.5	3.0–21.0	4.82
Fathead minnow			
12	7.3	2.0–13.0	2.95
17	2.4	0.5– 4.0	0.94
22	2.4	0.5– 5.0	1.21

Table 3. Effects of water hardness on induction time (time to loss of equilibrium in 50% of the fish) at 40 mg/L MS-222. Induction time differences are significantly greater in very soft water than in soft water for rainbow trout and fathead minnows. Induction times for carp were significantly greater than times for trout and minnows at all levels of water hardness ($P < 0.01$).

Species and water hardness[a]	Induction time (min)		
	Mean	Range	SD
Rainbow trout			
Very soft	4.5	1.0– 7.0	1.61
Soft	1.9	0.5– 4.0	0.94
Hard	3.8	1.0– 8.0	1.88
Very hard	5.5	1.0–11.0	2.68
Common carp			
Very soft	22.3	11.0–36.0	8.13
Soft	24.0	10.0–35.0	6.70
Hard	32.8	10.0–52.0	11.26
Very hard	43.8	11.0–73.0	16.62
Fathead minnow			
Very soft	9.0	2.0–19.0	4.56
Soft	3.8	1.0–10.0	2.41
Hard	7.3	2.0–14.0	3.22
Very hard	8.0	3.0–14.0	2.94

[a] See Table 1 for water characteristics.

Water hardness exerted a significant effect on mean induction times, but with important variations (Table 3). In rainbow trout and fathead minnows, the

times were significantly greater in very soft water than in soft water. For carp, mean induction time was 24.0 min in soft water and 22.3 min in very soft water. In all three species, resistance to MS-222 in very hard water was greater than in hard water. Induction times at all water hardnesses were significantly greater for carp than for rainbow trout and fathead minnows.

Increasing the stocking density from about 0.3 to 1.2 g/L increased resistance to the effects of MS-222 in all species (Table 4). Mean induction times at all stocking densities for rainbow trout were significantly less than times for carp. The times for fathead minnows were not significantly different from those for rainbow trout at any density.

The slopes for the regression equations for induction times with increasing temperatures, water hardness, and stocking density were all significantly different from zero. For all three species, the slope was negative for the temperature regressions (i.e., increased temperatures resulted in shortened response times). The slopes for regressions of water hardness and stocking densities were positive, indicating increasing resistance with increasing water hardness and stocking densities.

Table 4. Effects of stocking density on induction time (time to loss of equilibrium in 50% of the fish) at 40 mg/L MS-222 in soft water. Increasing average weight per test container for all species significantly increased mean induction times. Induction times for rainbow trout and fathead minnows are not significantly different ($P < 0.05$).

Species and stocking density (no. fish per tank)	Average weight/15 L (g)	Induction time (min)		
		Mean	Range	SD
Rainbow trout				
5	4.5	3.5	1.0- 8.0	3.01
10	9.0	4.5	3.0- 6.0	0.80
15	13.5	5.0	1.0-10.0	2.57
Common carp				
5	6.0	23.5	11.0-39.0	12.04
10	12.0	24.0	10.0-35.0	6.43
15	18.0	26.3	10.0-47.0	10.58
Fathead minnow				
5	4.0	6.3	4.0-10.0	2.58
10	8.0	7.3	2.0-13.0	2.95
15	12.0	8.5	2.0-16.0	4.00

Discussion

Results of the present study suggest that increasing the water temperature increases the efficacy of MS-222 and shortens induction times in rainbow trout, carp, and fathead minnows. The results of a number of thermal stress experiments have demonstrated that increases in gill ventilation and cardiac rates occur as acclimation temperatures are increased over a short time span (Roberts 1973; Fry 1967; Tsukuda 1961). Phillips et al. (1957) found that MS-222 caused increased cardio-ventilatory activity independent of temperature and that this increased activity was accompanied by significant increases in ionic permeability of the gills.

The influence of water hardness on the efficacy of MS-222 may be species-dependent and could be a function of the pH buffering capacity of hard water. Houston et al. (1970) and Brooke (1964) demonstrated different rates of calcium metabolism by fish in water of different hardnesses and different temperatures. Significant alterations in the blood constituents of fish exposed to MS-222 were demonstrated by Soivio et al. (1977) and Hattingh (1977). The combined effects of the alterations at different water hardnesses significantly increased hematocrit values, blood lactate concentrations, and concentrations of plasma K^+ and Mg^{++}.

The effects of pH on the anesthetic properties of MS-222 were described for mullet by Sylvester (1975) and rainbow trout by Black and Conner (1964) and Wedemeyer (1970). Induction times were reduced at either low pH (< 5) or high pH (> 7). These workers believed that this effect was due to a pH-related irritation of gill membranes by solutions of MS-222 (at 80 mg/L, pH = 4). Wedemeyer (1970) indicated that MS-222 solutions adjusted to pH 7 consist almost entirely of the free base form of ethyl *m*-aminobenzoate and that its efficiency is unaffected by the adjustment. He further concluded that neutralized MS-222 reduced the stress of the anesthetic as reflected by liver function, renal and gill function, and pituitary action.

Increased oxygen concentrations have been shown to lengthen anesthetic induction times in mullet (Sylvester 1975). This increase may be the result of a decrease in the stress effects of the anesthetic at higher oxygen levels. Alternatively, the relative action of MS-222 could be enhanced by a reduction in dissolved oxygen concentration in bath solutions.

The reasons for increasing resistance to MS-222 with increased numbers of fish per test remain obscure, although at higher stocking densities, interacting effects of reduced dissolved oxygen and increased metabolites may affect the drug's efficacy. The effects of factors such as exposure time, recovery time, species differences, and handling characteristics all affect toxicities and drug response curves (Jones 1964). Social and behavioral adaptations to toxicants and anesthetics in fish have not been thoroughly studied. Fish have the ability to neutralize and eliminate poisons to some extent, and whether this ability can be altered by the proximity of increased numbers of the same species is not known.

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Accepted 20 January 1982

Prophylactic Treatment of Elvers for *Trichodina* Infestation[1]

In Japan, where intensive culture of *Anguilla japonica* has been a commercial success since the late 1800's, damage occurs from regular outbreaks of *Trichodina*, a ciliated protozoan that lives on the skin and gills of many species of fish. Similar problems may arise as culture of American eel (*A. rostrata*) expands in the United States. Culture ponds are usually stocked with glass or elver stages of American eel collected from coastal rivers during their annual migrations. In 1981, 90% of the elvers sampled from Cooper River, South Carolina, were infested with *Trichodina*. Under conditions of stress, several species of *Trichodina* may become pathogenic, interfering with feeding and respiration of small fish such as glass eels and elvers (Crane and Eversole 1980; Rickards 1978; Rogers 1979).

Formalin is most often recommended for control of *Trichodina*, and has been used successfully in experimental eel culture (Eversole, unpublished data). Unfortunately, formalin (37% formaldehyde) is not registered for use with food fish (Schnick et al. 1979). Salt (NaCl) and potassium permanganate ($KMnO_4$), both of which are registered for food fish use, are alternate controls for *Trichodina* (Amlacher 1970; Hoffman and Meyer 1974; Reichenbach-Klinke and Elkan 1965; and others). At least 21 different application rates of NaCl or $KMnO_4$ are referenced in the literature for control of *Trichodina* on freshwater fish. However, none specified the use of either chemical on American eels.

Amlacher (1970) suggested that short-duration baths (or dips) are more effective and safer than longer term treatments for skin and gill infestations of protozoans like *Trichodina*. Short-term baths or dips are easily administered to eels when they are collected from rivers or just before stocking. We tested the efficacy of NaCl and $KMnO_4$ as *Trichodina* control agents, selecting times and levels for short-term baths from the range of application rates recommended by Amlacher (1970), Hoffman and Meyer (1974), and Reichenbach-Klinke and Elkan (1965).

Tests were conducted in glass jars, each containing 10 L of deionized water reconstituted to 40–48 mg/L hardness, 30–35 mg/L alkalinity, and pH 7.2–7.6, as recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Water was continuously aerated and temperatures averaged 21.2°C during tests. *Trichodina*-infested elvers (85–117 mm TL) collected from the Cooper River in February 1981 were assigned to test jars at random. Ten elvers were used in each of six $KMnO_4$ treatments plus a control; eight elvers were assigned to each test vessel in four NaCl treatments. Bath treatments were used at the application rates listed in Table 1. After treatment, elvers were rinsed and then placed in reconstituted water for a 96-h observation period.

Following the observation period, elvers were placed individually in formalin baths to dislodge any remaining *Trichodina* (Crane and Eversole 1980). Settleable materials in formalin baths were subsampled with a pipette, *Trichodina* were counted in eight well formed drops from this subsample, and the mean number of *Trichodina* per eel was calculated for the controls and for each treatment. The percentage of infested elvers (percent occurrence) and the relative abundance of *Trichodina* per eel (percent intensity) were calculated to evaluate the relative efficiency of each chemical treatment. Percent intensity was calculated for each treatment by dividing the mean number of *Trichodina* per eel by the mean number of

Table 1. Laboratory trials of potassium permanganate and salt treatments of elvers infested with Trichodina.

Treatment and concentration (mg/L)	Time (min)	Occurrence (%)	Intensity[a] (%)
Control	30	100	100
Potassium permanganate			
5	30	20	4
10	30	0	—
50	10	40	7
100	10	0	—
500	0.5	0	—
1,000	0.5	0	—
Salt			
10,000	10	100	71
10,000	30	25	58
30,000	10	50	49
30,000	30	50	9

[a] Percent intensity based on the mean number of *Trichodina* per control eel as 100%.

[1] Technical contribution 1988 of the South Carolina Agricultural Experiment Station. Financial support for this investigation was provided by the South Carolina Sea Grant Consortium and the South Carolina Experiment Station (Regional Research Project S-168).

Trichodina per control eel. Subsamples from control eels averaged 5.6 *Trichodina* per eel (range 1–18).

No mortality was observed during the 96-h test. Potassium permanganate was more effective than salt in controlling *Trichodina* (Table 1). Effective concentrations of $KMnO_4$ included 10 mg/L for 30 min, 100 mg/L for 10 min, and 500 and 1,000 mg/L for 0.5 min. Short exposures at high concentrations of $KMnO_4$ appeared to control *Trichodina* more effectively than long exposures at low concentrations. None of the NaCl concentrations tested totally controlled *Trichodina* (Table 1). According to Reichenbach-Klinke and Elkan (1965), NaCl baths and dips may need to be repeated several times to be successful.

In tolerance tests, glass eels and elvers taken from fresh water and exposed to NaCl concentrations of 20–45 g/L showed signs of stress immediately (Hinton and Eversole 1978, 1979). Hinton and Eversole (1978, 1979) also observed 100% mortality when glass eels and elvers were exposed for 12 h to 30 and 35 g/L NaCl. Caution must be exercised when NaCl is used at the concentrations recommended for control of *Trichodina* to treat eels held in fresh water, whether in repeated baths or in long-term exposures.

Hinton and Eversole (1978, 1979, 1980) reported no mortality among glass, elver, and yellow stage eels during a 10-min exposure to 100 mg/L $KMnO_4$ or in a 96-h post-treatment period. Mortality was observed in concentrations of $KMnO_4$ as low as 5 mg/L when glass eels were exposed for 12 h and elvers for 24 h (Hinton and Eversole 1978, 1979). Mortality reportedly occurred among yellow stage eels when applications exceeded 15 mg/L for 24 h (Hinton and Eversole 1980). Calculated 96-h LC50 values for $KMnO_4$ with glass, elver, and yellow stage eels were 3.06, 4.86, and 21.60 mg/L, respectively (Hinton and Eversole 1978, 1979, 1980). Considering these and present test findings, we believe treatment at 100 mg/L $KMnO_4$ for 10 min should be safe and effective for all stages of American eel.

Glass eels and elvers should be examined to determine if prophylactic treatment is necessary to prevent introduction of *Trichodina* before recently collected stocks are combined with culture stocks. Unless and until formalin is approved for use with food fish, $KMnO_4$ appears to be the registered chemical most effective in the control of *Trichodina* infestations of eels.

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Accepted 18 February 1982

Chamber for Maintaining Adult *Daphnia* and Separating the Young[1]

Daphnia have long been known to be sensitive to waterborne pollutants (Anderson 1950), and their use as a test animal for water quality assays has been generally accepted (American Public Health Association [APHA] et al. 1976; Kenaga and Moolenaar 1979; Leeuwangh 1978). The use of newborn daphnids (no more than 24 h old) has been recommended for both acute and chronic toxicity tests (APHA et al. 1976; Adema 1978), and a method for the mass rearing of *Daphnia* in large funnels and the separation of newborns from the adults by means of the daphnid's phototactic response was demonstrated by Parker and Dewey (1969). We present here an improved culture chamber (Fig. 1) that has been successfully used for the production of *D. pulicaria* Forbes of known age (Bertram 1980). We have also successfully maintained *D. galeata-medotae* Birge and an unidentified chydorid in this vessel.

The basic features of the modified system include an opaque chamber made from polyvinyl chloride (PVC) pipe 15 cm (6 in.) in diameter, a PVC ring and collar that hold a piece of Nitex brand plankton netting, a PVC funnel fitted with a wide-bore ball valve, a clear glass wide-mouth jar that screws onto a cap which has been affixed below the valve, and a light source. The whole system is filled with water and the valve is left open. An inflow line entering below the screen and a standpipe outlet above the screen allow for the continuous flow of the water through the chamber. The volume of water in the chamber is controlled by the height of the standpipe. Up to 3 L may be held in the chamber above the screen. The plankton netting for the screen is chosen with apertures of a size such that adults cannot pass through but newborn young can. An opaque cover ensures darkness in the chamber except for the light entering through the collection bottle when the valve is open.

Daphnia attempt to swim toward the source of light below the glass jar. Adults are stopped by the netting, but the young continue to swim into the collection jar. The jar and its contents of young daphnids may be removed at any time.

We offer the following operating suggestions:

• Air may become trapped under the screen when the chamber is first filled with water, particularly with screens of mesh less than 0.2 mm. The air may be quickly removed with a pasteur pipette attached to an eyedropper bulb.

• When the chamber is used as a static water system, we recommend that the water inlet line be converted to an air line that ends above the screen, and air flow be provided to create a bubbling rate of about 2/s.

• The screen tends to become clogged with accumulated exuvia, and the system ceases to be effective after about 2 weeks at 15°C. The water with the remaining

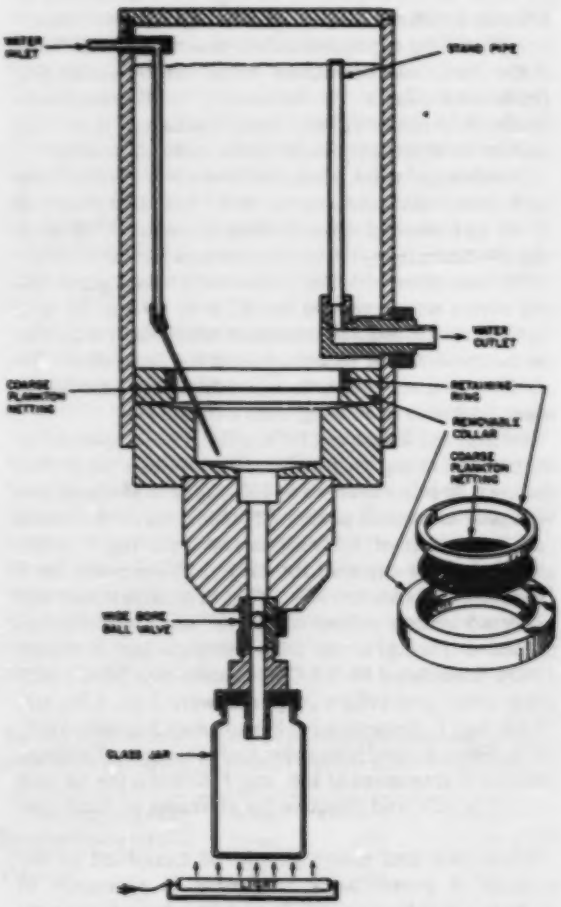

Fig. 1. Diagram of a chamber used for the culturing of adult Daphnia *and the separation of their newborn offspring.*

[1] Contribution No. 228, Center for Great Lakes Studies, The University of Wisconsin-Milwaukee.

adults may then be drained and the apparatus disassembled for cleaning. The cover lifts off, the water inlet is released from its set screw, the water outlet is removed from its O-ring seal, and the collar for holding the screen is lifted out. After cleaning, the chamber may be re-assembled, refilled, and restocked with the same adults or with a fresh stock.

Acknowledgments

We thank Robert Scott for improving our original designs and for manufacturing the chambers, and Ratko Ristic for drawing the figure. This work was funded in part by the University of Wisconsin Sea Grant College Program under a grant from the Office of Sea Grant, National Oceanic and Atmospheric Administration, U. S. Department of Commerce, and by the State of Wisconsin.

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Accepted 20 January 1982

Techniques for the Capture and Handling of Atlantic Mackerel With Special Reference to the Use of Quinaldine

Atlantic mackerel (*Scomber scombrus*) were maintained in the laboratory for more than a year while a study on the energetics of their feeding, metabolism, and growth was completed. In addition to the problem of capturing and establishing the fish in the laboratory, it was periodically necessary to transfer fish temporarily from holding tanks to a top-loading balance for weighing and to a respirometer for determination of O_2 consumption rates. Techniques involving the use of the anesthetic quinaldine (2-methyl quinoline) were developed to effect these operations safely and efficiently.

Initial attempts to bring mackerel into the laboratory met with little success. The fish were dipnetted from a commercial trap net and transported in aerated seawater to the laboratory. Here they were transferred, again by dipnet, to holding tanks 2.4 m (8 ft) in diameter. Bruise-like discolorations began to develop on the body 3–4 days after capture and these lesions grew steadily worse until eventually most of the epidermis sloughed off; at this point death was imminent. Such delayed mortality has been attributed to osmoregulatory difficulty (often aggravated by physical damage to the skin), oxygen deficit due to poor ram ventilation at reduced swimming speed, and lactic acid buildup—all induced by the stress of capture and handling. These effects of stress have been discussed in detail by Mazeaud et al. (1977).

Pawson and Lockwood (1980) demonstrated that the pO_2 in blood moving away from the gills of

mackerel was only slightly depressed by slowing down the rate of movement of the fish through the water. They also recorded high levels of lactic acid in the blood after stressful activity, but these returned to normal levels within 8–12 h after the fish were allowed to swim quietly again. These authors also found that in mackerel that had been roughly handled or kept in overcrowded enclosures (greater than 50 fish/m^3), the levels of sodium and cortisol in the blood tended to rise with time and these rises were associated with the eventual death of the fish; this was attributed to dehydration in a hypertonic environment following damage to the skin.

Since the osmotic pressure of the blood of marine teleosts is 35–45% that of seawater (Black 1957), I attempted to alleviate osmotic stress by placing newly captured mackerel in seawater of appropriately reduced salinity. At a salinity of 15 °/₀₀, cumulative mortality was reduced from 95 to 50%. At this point it became clear that further reduction in mortality would only be made by eliminating physical damage to the skin of the mackerel. Accordingly, I altered the method of capture.

Mackerel were obtained, as previously, from a commercial trap but were angled with a barbless hook before the trap was pursed, rather than being dipnetted after the pursing. The angler quickly swung a hooked fish aboard the boat, grasped the shank of the hook firmly with one free hand, and with a sharp downward, twisting motion of the wrist dropped the fish directly into a round tank of water, containing a sedative level of anesthetic (1 mg/L vol/vol). Thus, other than a minor puncture wound caused by the hook, the mackerel was undamaged and the body epidermis remained intact.

Quinaldine, the anesthetic selected, is inexpensive and highly effective at very low concentrations; its toxicity is low and fish recover from anesthesia rapidly (Muench 1958; Bell 1964). Since quinaldine is not readily soluble in water, it was first mixed with acetone (in which it is moderately soluble) to obtain better dispersal (10 parts of acetone to 1 of quinaldine). At the laboratory, the mackerel were transferred to the holding tank (2.4 m in diameter) after the anesthetic level was increased from 1 to 2 mg/L. At this concentration schooling behavior ceased and there was a slight loss of equilibrium; the fish displayed little or no avoidance reaction and swam into a submerged plastic bucket in which they were carried between tanks, at all times completely surrounded by water.

To maintain any particular level of anesthesia, it was necessary to add more quinaldine after about 15 min because of its tendency to coalesce into droplets that sink. Judging by the strong odor in the vicinity of its use, there must also have been some evaporative loss.

Anesthetization was an essential part of the weighing procedure during growth studies of mackerel. The group of fish to be weighed were subjected to a concentration of 2–3 mg/L of quinaldine and were then transferred, one at a time, to a small tank containing 4–6 mg/L of the anesthetic. When movement ceased, the fish was lifted in a soft plastic sling (wetted and pre-weighed) to a top-loading balance and weighed. Then, still in the sling, the mackerel was carried to a third tank of pure seawater, where it was resuscitated by directing a stream of water into the mouth and over the gills.

At 12–17°C complete anesthetization (4–6 mg/L) required 5–10 min. In water at 6–8°C the process was more rapid (2–4 min) and less quinaldine was required (hence the range in stated dosages). Williamson and Roberts (1981) found that cooling also enhanced the effect of MS-222 (tricane methanesulfonate) on dogfish (*Scyliorhinus canicula*).

The weighing and resuscitation of mackerel took about 1 min; complete recovery was swift (2–3 min), and the fish would feed immediately. The fish used in these studies varied in size from 250 to 450 g (fork length, 290–380 mm); within this range, susceptibility to quinaldine did not appear to be size-dependent.

During tagging procedures fish are subjected to extremely stressful conditions. From the present study and the work of Pawson and Lockwood (1980), it appears that mackerel are particularly delicate and exceptionally prone to the effects of stress. It is therefore highly probable that the usual tagging procedures for mackerel lead to high subsequent mortality. Tagging returns have indeed been poor; expressed as a percent of total mackerel tagged, recaptures of 0.2, 0.7, and 2.0% (Parsons and Moores 1974; Moores et al. 1975; MacKay 1967, respectively) have been reported. Many of these recoveries were less than 1 week after tagging, before the full consequences of the handling stress would have been realized.

On completion of the energetics study, I therefore further tested the handling technique for mackerel with a tagging experiment. Using the previously described weighing procedure, I tagged eight fish with Floy "spaghetti tags" (Dell 1968). The tags were anchored in the dorsal musculature immediately posterior to the first dorsal fin. These fish and a like number of untagged fish were maintained in the laboratory under identical conditions. The growth of all fish was monitored monthly by weighing and after 6 months there was no significant difference between the weights of the two groups. Also there were no differences between the swimming and schooling behavior of tagged and untagged fish. At the end of the period of assessment the tags were still secure and causing no obvious damage or irritation to the mackerel.

The levels of anesthesia described here for tranquilizing (1 mg/L), transferring (2–3 mg/L), and weighing (4–6 mg/L) mackerel correspond to Mc-

Farland's (1959) Stage I plane 1, Stage II plane 1, and Stage II plane 2, respectively, in terms of reaction to visual stimuli and state of equilibrium. Other criteria used by McFarland in his definitive paper on behavioral and physiological effects of anesthetic on killifish (*Fundulus* spp.) and opaleye (*Girella nigricans*) cannot be applied to mackerel; he detailed color changes and variations in swimming behavor, including fin positioning, none of which occur in mackerel.

The dosages of quinaldine required to anesthetize mackerel were much lower than those reported for freshwater fish (Schoettger and Julin 1969). Other saltwater species that I have anesthetized successfully (Atlantic herring [*Clupea harengus harengus*], gaspareau [*Alosa pseudoharengus*], and Peruvian anchovy [*Engraulis ringens*]) required the same dosages as mackerel for any particular level of anesthesia. Similar concentrations were used by Gibson (1967) on five species of marine fish. Why marine teleosts appear to be more sensitive to quinaldine than freshwater fishes is unclear. Although saltwater fish must drink water to maintain osmotic balance, the amounts are not enough (Krogh [1939] reported 40 to 225 mL of seawater per kilogram of weight was swallowed per day by eels [*Anguilla*] and sculpin [*Myoxocephalus*]) to allow sufficient absorption through the stomach, especially during the short period required for the anesthetic to take full effect. Nevertheless, it is probable the cause is more physical than physiological, since there is no reason to suppose quinaldine's action of depressing the central nervous system, notably the respiratory center (Bell 1964), should be any different in marine and freshwater species.

It seems clear that any tagging program involving delicate species like mackerel should include the use of anesthetic. If an anesthetic is not used, an empirical estimate of tagging mortality must be made if subsequent results are to have any credibility.

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Accepted 3 February 1982

Observations on Salinity Tolerance of Striped Bass x White Bass Hybrids in Aquaria

The original male white bass (*Morone chrysops*) and female striped bass (*Morone saxatilis*) hybrid was first produced (Stevens 1965) at Moncks Corner, South Carolina, and has been accepted as a freshwater sportfish in the southern United States. The reciprocal hybrid was first reported by Bayless (1968). Both *Morone* hybrids are produced and stocked by the Florida Game and Fresh Water Fish Commission.

Striped bass are anadromous fish and therefore euryhaline. According to Moyle (1976), white bass have an unknown salinity tolerance but can tolerate brackish water. Bayless (1972) reported that larvae of the original hybrid grew best at a salinity of 10.5 °/₀₀ and survived best at 17.5 °/₀₀. Sport fishermen have reported catching hybrids in areas of Escambia Bay, Florida, which reach a salinity of approximately 26 °/₀₀ (De Witt Bass, Florida Game and Fresh Water Fish Commission, personal communication). The present study was undertaken to provide preliminary observations concerning salinity tolerance of reciprocal and original *Morone* hybrids, since the hybrids stocked in Florida frequently have access to brackish water.

Original and reciprocal hybrids spawned at Richloam Fish Hatchery, Webster, Florida, were used. They were transported to the Non-Native Fish Research Laboratory, Boca Raton, Florida, at a total length (TL) of 25–40 mm and reared to about 100–125 mm TL in a 0.01-ha pond stocked with mosquitofish (*Gambusia affinis*).

Biological substrate filters were placed in six 300-L glass aquaria, and each aquarium was stocked with eight hybrids. Sets of three tanks were used for each type of hybrid. In each set one tank was used as a freshwater control, and two were converted to full-strength seawater (salinity = 36 °/₀₀) by increasing salinity 6 °/₀₀/day with Instant Ocean.

Each morning at 0800 h salinity (American Optical Refractometer), dissolved oxygen (Yellow Springs Instrument Co. meter-51A), hydrogen ion concentration (Corning pH meter-610), and temperature (mercury thermometer) were measured, and the number and behavior of hybrids and mosquitofish were recorded. Mosquitofish (five per hybrid) were then placed in each tank, and 30 min later Instant Ocean was added. Salinity, temperature, and fish behavior were again noted at 1200 and 1600 h.

After 4 days in full-strength seawater, hybrids from one seawater tank for each type of hybrid were exchanged with the same type of hybrids from the freshwater control tanks without acclimatization. The second seawater tank for each type of hybrid was left at a salinity of 36 °/₀₀. Formal observations were continued for 3 days, following which fish were left in these tanks for 2 more days, but not closely observed. All hybrids were then directly transferred to a 3,400-L circular freshwater tank.

Eight hybrids of each type survived a 6-day acclimation to a salinity of 36 °/₀₀, 7 days at that salinity, and then instant transfer to fresh water. Other hybrids survived instant transfer from fresh to saline water at 36 °/₀₀, where they were maintained for 3 days and then returned directly to fresh water. While salinities were being increased two test fish died after jumping out of tanks and one control fish died from an unknown cause. All hybrids transferred to the circular tank at the conclusion of the experiment remained healthy an additional 2 weeks, at which time they were stocked in ponds.

Regardless of salinity, hybrids tended to swim in a school near the bottom. During gradual hybrid acclimation to salinity, mosquitofish were always consumed within 30 min of stocking. Mosquitofish that were stocked 5 min after exchange of hybrids between fresh and sea water were immediately chased and consumed (within 10 min) by hybrids.

Mean water quality values were similar among all tanks throughout the experiment: dissolved oxygen 8.5 ± 0.2 mg/L, pH 6.4 ± 0.7, and temperature 23.8 ± 0.2°C.

These observations suggest that both original and reciprocal *Morone* hybrids may be euryhaline and able to withstand instant transfer between fresh and salt water. Therefore, hybrids probably can migrate from fresh water to brackish or full-strength sea water. Consequently, hybrid salinity tolerance should be considered when stocking areas connected to estuarine or saltwater habitats.

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Accepted 3 February 1982

Influence of Formalin on Size of Walleye Eggs

The size of eggs is of considerable importance in relation to subsequent development of fish (Dahl 1918–19; Bagenal 1969; Nikolsky 1969; Fowler 1972; Gall 1974; Pitman 1979). However, the ususal practice in fecundity studies is to make measurements on eggs preserved in 10% formalin (often for a long time) without considering the possible effects of the preservative on egg size (Wolfert 1969; Stauffer 1976; Emery and Brown 1978). The only fecundity study that described preservative effect on fish eggs that I could find in the literature was done by Taube (1976). He reported little size change in eggs of brown trout (*Salmo trutta*) that were measured at 2–4 months and 2 years after preservation in 10% formalin. Conversely, in another test he reported that fresh brown trout eggs increased in volume after preservation for 11 and 25 days.

It was uncertain, therefore, if measurements of preserved eggs, done as part of a walleye (*Stizostedion vitreum vitreum*) fecundity study I conducted, accurately represented the diameter of fresh walleye eggs. A sample of fresh eggs from the ovary of a 50.5-cm walleye collected on 26 April 1980 was measured to the nearest 0.03 mm. The eggs were then placed in 10% unbuffered formalin and 30–36 eggs were measured at intervals of 5 days to 20 months after preservation. Over this period mean walleye egg diameter varied only 0.03 mm (1.61–1.64 mm) on 17 separate occasions, indicating no change in egg size as a result of the preservative.

Acknowledgments

I thank M. H. Hoff, who did most of the egg measurements. This research was supported in part by Federal Aid in Fish Restoration funds under Wisconsin Dingell-Johnson Project F-83-R.

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Accepted 20 January 1982

Two-stage Collector for Floating Eggs

Floating eggs from cultured marine fishes are often obtained by tank spawning. The capacity of these tanks usually ranges from 2 to 20 m^3 and they may contain only a pair or as many as 50 brood fish. Fertilized eggs are collected by netting them from the water surface or by diverting the outflow of the tank through a plankton net or basket. Such methods have been used in work with Dover sole (*Solea solea*; Kingwell et al. 1977), red sea bream (*Pagrus major*; Fujita 1979), sea bass (*Dicentrarchus labrax*; Girin 1979), gilthead bream (*Sparus aurata*; Gordin and Zohar 1978), and silvery black porgy (*Acanthopagrus cuvieri*; Hussain et al. 1981).

When the first method (netting) is used, it is often necessary to stop the flow of air and water into the tank to allow the eggs to collect on the water surface before hand netting. This requirement limits the density at which spawners can be held, since fish in a densely stocked tank would rapidly exhaust the available oxygen. Moreover, turbulence caused by fish movement in the tank tends to redistribute the eggs in the water column, so that collection is incomplete. To collect most of the eggs when the second method (outflow divergence) is used, a large water flow (typically, 20–50 L/min) must be passed through a collector made of fairly fine netting (300–800 μm) for several hours, which often leads to egg damage or net clogging, overflow, and egg loss.

By siphoning the eggs from a primary collector into a second net collector or basket (Fig. 1), such problems are reduced. Eggs are initially caught in the first collector and then carried by a small siphon to the second collector, where they accumulate and are kept in gentle motion by the small flow. If the flow is about 1 L/min and the water volume contained in the first collector is about 10 L, the average residence time of eggs in the first collector will be only about 10 min. The flow in the first collector is usually the entire water flow for maintaining the spawning tank (typically from 20 to 50 L/min), whereas in the second collector the flow may be as little as 0.2 to 1 L/min. Thus, any overflow occurring in the first collector will not cause the loss of the bulk of the eggs. Furthermore, eggs are exposed to the full force of the main water flow for minutes, rather than hours, and spend most of the collection period in the second collector, where conditions are more suitable for incubation.

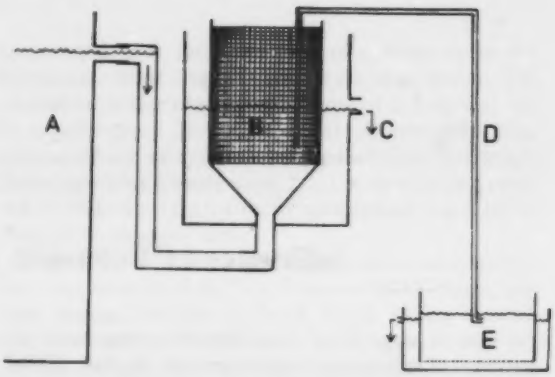

Fig. 1. Diagram of the two-stage egg collector: A, spawning tank (capacity of several cubic meters); B, first egg collector (capacity, 10 L); C, overflow outlet; D, siphon tube; and E, second egg collector. Flow from A to B is at a rate between 20 and 50 L/min, whereas flow from B to E is between 0.2 and 1 L/min.

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Accepted 20 January 1982

Golden Shiner Virus: Effects of Stocking Density on Incidence of Viral Infection

The golden shiner virus (GSV) was first isolated in 1977 from golden shiners (*Notemingonus crysoleucas*; Plumb et al. 1979). The viral particle has biochemical and biophysical properties similar to those of infectious pancreatic necrosis virus (IPNV; Malsberger and Cerini 1963; Cohen et al. 1973), however, the cytopathic effect in cell cultures of the fathead minnow (FHM — *Pimephales promelas*), growth characteristics, and serological properties separate GSV from IPNV (Schwedler and Plumb 1981). Although the virus was originally isolated from pond-raised golden shiners and low mortality was associated with the virus (Plumb et al. 1979), it has caused significant losses when infected fish were crowded in holding tanks. The objective of the present study was to determine the relation between population density and the incidence of GSV infection under natural exposure conditions.

During September 1979, 36 golden shiners (average, 5 g) were removed from a production pond (temperature, 30° C), pithed, iced immediately, and returned to the laboratory where they were assayed (within 24 h) for parasitic, bacterial, and viral pathogens. Concurrently, 750 fish were removed from the same pond and transported live to Auburn University in water containing 0.25 mg/L of Furanace. The fish were placed in two 200-L tanks at densities of 6.25 and 12.5 g/L, respectively, and held for 8 days under static, aerated conditions at 25–28° C. At 48-h intervals, 10 live fish were selected at random from each tank and individually assayed for parasitic, bacterial, and viral pathogens. Water samples from each tank were assayed for GSV at 24-h intervals. Although moribund and freshly dead fish were also assayed for parasitic, bacterial, and viral pathogens, they were not included as part of the regular samples.

Bacterial and parasitic examinations were made by routine diagnostic procedures. For virus isolation individual fish were minced with scissors and triturated for 2 min in a mortar and pestle. A 0.5-g sample of the slurry was diluted 1:10 in Hanks' balanced salt solution, sonicated at 100 kHz at 300 W (acoustical) for 1 min, centrifuged at room temperature for 15 min at 300× *g*, and clarified by using a 0.45-μm membrane filter. The filtrate was placed in triplicate wells (0.1 mL/well) of 2-cm² multiwell plates containing 90–100% confluent monolayers of FHM cells (Gravell and Malsberger 1965). Unused filtrate was stored at 4° C until needed. Supernatant from each well was blind-passed to a 24-h culture of FHM cells 3 days after inoculation. The original cultures and their respective blind passages were observed daily for cytopathic effect. When a sample was positive, the original 1:10 dilution was titered by inoculation of triplicate microtiter wells of FHM cells with serial 10-fold dilutions of the virus suspension. Tissue culture infectious doses (TCID 50/mL) titers were calculated by the Reed-Muench method (Reed and Muench 1938).

Golden shiner virus was detected in 2.8% (1 of 36) of the golden shiners removed from the pond. After 48 h virus was detected in 5 of 10 fish samples from the high-density population (12.5 g/L). The percentage of infected fish decreased until 196 h, when no GSV was detected (Table 1). In contrast, GSV was detected after 48 h in only 1 of 10 fish from the low-density golden shiner population (6.25 g/L). No GSV-infected fish were detected from the low-density stocking at 144 h.

The titer of GSV in the infected fish assayed immediately after removal from the pond was $10^{3.83}$ TCID 50/g of fish. Titer in infected fish removed from the low-density population was $10^{2.5}$ TCID 50/g of tissue at 48 and 96 h (Table 1). The mean titer of infected fish from the high-density tank at 48 h was $10^{3.3}$ and at 96 h $10^{2.85}$ TCID 50/g of tissue. At 144 h, the titer of the single infected fish from the high-density population was $10^{2.16}$ TCID 50/g. Golden shiner virus was isolated from water in the tank with high fish density and the titer ranged from $10^{2.5}$ to $10^{2.83}$ TCID 50/mL. No virus was isolated from the water with low fish density.

Several factors could be responsible for the increased incidence of viral infection from 2.8% in the pond population to 50% in the high-density population of golden shiners. A large percentage of the fish in the pond could have been infected with virus but not detected by the methods used. Physiological changes related to crowding and possibly other environmental factors may have potentiated the increase in virus to detectable levels. An increase in GSV incidence mediated by crowding would seem logical, since the effects were less dramatic in fish held at the lower density. In fact, GSV could be isolated from the water of the high-density tank after 48 h, suggesting that virus was being shed. Apparently the virulence of GSV is relatively low. Mortality of 5% occurred at both population densities, even though an increased inci-

Table 1. Golden shiner virus (GSV) titers in living golden shiners held at stocking densities of 6.25 and 12.5 g/L.

Time (h)	Fish Density					
	6.25 g/L			12.5 g/L		
	GSV positive[a] (%)	Titer (TCID 50/g)		GSV positive[a] (%)	Titer (TCID 50/g)	
		Range	Mean		Range	Mean
48	10	-	10	50	$10^{2.5}$ - $10^{3.83}$	$10^{3.30}$
96	10	-	10	33	$10^{2.16}$ - $10^{4.6}$	$10^{3.85}$
144	0	-	-	10	- -	$10^{2.16}$

[a] Ten fish were assayed from each stocking density at each sample period.

dence and elevated level of viral infection occurred in the fish at the higher population density. No bacteria were isolated from any of the sampled fish, nor were any parasites detected.

Acknowledgment

This research was supported by the Southeastern Cooperative Fish Disease Project and Sport Fish Restoration Funds.

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Accepted 10 February 1982

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Technique for Converting a Beach Seine for Operation by One Person

Sampling schedules for inshore fishes, both marine and freshwater, may not respect the timetables of available field assistants. At such times, it is convenient to have an alternative to cancelling the proposed field trip. I have devised a method by which a two-man beach seine can be quickly and inexpensively converted to a form operable by one person. Conversion (or reconversion) can be accomplished in about 5 min; initial costs are less than $5.

A conventional beach seine, preferably one with a bag, and up to 9 m long, can be used. The principle of construction is similar to that of a beam trawl, with the beam serving to keep the seine mouth open during operation.

The seine is laid out on the beach where the collection is to be made, and the wing ropes short-tied so that the loop connecting the float line to the lead line stretches about 1 m in front of the net (Fig. 1). If a pole seine (a seine with a vertical pole at the end of each wing) is being used, a length of polypropylene line (8 mm in diameter) is tied to the pole to form a loop as described above. The loop should be knotted so that the knot is 20–30 cm off-center toward the lead line and incorporates another loop of sufficient diameter to accommodate a piece of wood 5 x 5 cm.

A "beam" is then inserted. The beam consists of two pieces of lumber 5 x 5 cm, bolted together at the center, with the ends protruding through the loop knots. The two halves of the beam are left overlapping by 30–40 cm in the middle and then fastened together with bolts and wingnuts through pre-drilled holes at each end of the overlapping section. For a small seine, a single beam would suffice. About 30 cm from each end of the beam four nails (10 cm) are hammered part of the way into the wood. When the beam ends have been inserted into the knots on the loops, the protruding nails prevent the loops from slipping along the beam and closing the seine mouth. Beam length will vary with the size of the seine used; however, it should be long enough to keep the seine mouth open to a sufficient degree, and short enough to maintain a "half-moon" shaped bag in the seine during operation. For a 9-m seine, a beam 3–4 m long is satisfactory.

To complete the seine conversion, one end of a 10- to 15-m length of 8-mm polypropylene line is tied to the center of the beam, and the other end is formed into a pulling handle. When the seine is pulled, the wooden beam floats and does not disturb bottom fish in the area. The off-center loop knots act to keep the lead line slightly ahead of the float line — a preferred condition for a beach seine.

If the seine were to be pulled in a straight line, the net would sweep an area in which the operator had disturbed the fish. This procedure appears to render the catch less representative, particularly with respect to certain pelagic fish species. It is therefore preferable that the operator walk in an S-shaped path, although the path of the net may continue to be linear. Note that the operator is not restricted to sampling a circumscribed area, since the net is not tied to shore in any way.

More than 20 trials of the one-man seine have produced catches comparable in numbers and species to those derived from parallel, two-man hauls of equivalent cross-sectional area. A series of five concurrent 15-min paired sets on a sand-bottomed estuary produced a similar species catch in both one-man and two-man hauls (8 species in common and 1 uncommon species unique to each). The catch per species summed over all of the sets did not differ by more than one

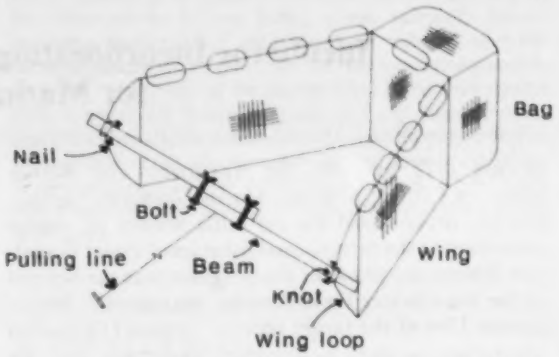

Fig. 1. Schematic diagram (not to scale) of a two-man beach seine converted for use by one person.

standard deviation between the one-man and two-man seines. The efficiency and size-selectivity of the one-man net appears to be similar to that of a conventional seine of equivalent cross-sectional area. The increased bag size (due to the large net curvature) retained captured fishes well, compensating for a pulling velocity only about 75% that of a two-man haul. Fish in both the water column and on the bottom were captured.

The converted seine is useful and appropriate in many, although not all, typical seining locations. I have successfully used a 9-m seine over both sand and mud bottoms in estuarine and marine locations. The energy required to pull such a large net (of 0.63-cm stretch mesh) for three 15-min sets is enough to provide a reasonable (but not debilitating) workout for a person of average strength. Under adverse conditions (such as in surf or through dense aquatic vegetation) the use of a smaller seine would be advisable. However, the advantage of having a one-man seine that is portable and is easily assembled and dismantled makes most fish-collecting forays of this type independent of field assistance.

Acknowledgment

This project was supported by a Natural Sciences and Engineering Research Council of Canada grant to Norman J. Wilimovsky.

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Accepted 3 February 1982

Incubator Incorporating Air-powered Water Flow for Marine Fish Eggs

During 1978 to 1980, the Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, investigated the early life history of species important to the recreational fisheries of Puget Sound. The laboratory phase of the program was carried out at the Manchester Field Station, Manchester, Washington. One of the target species, lingcod (*Ophiodon elongatus*), spawns a demersal egg mass with an average incubation period of 7 weeks (Miller and Geibel 1973). Portions of egg masses were collected in the field, brought into the laboratory, and incubated for use in a variety of embryological and larval investigations.

Experience in the laboratory has shown that adequate ventilation of adhesive eggs is necessary to assure proper embryo development. Among the criteria for the design of an incubation device for such eggs were that it must provide water flow velocities sufficient to thoroughly ventilate egg masses as large as 100 mL while avoiding undue agitation, and that it must operate in either open or closed systems and remain functional during pumping or power failures. (Since the air compressors at the Manchester field station are coupled with an auxillary propane-powered generator, uninterrupted service can continue during power failures.)

Incubators were constructed of clear acrylic tubing and sheeting, cemented with acetone. The major components of each incubator unit are a central chimney, which provides the airlift; two water intake chambers; and two incubator baskets which hold the eggs (Fig. 1). The chimney is fitted with a glass-bead airstone at its base and two intakes (180° apart) are connected to the chimney just below the airstone. Both the bottom and removable lid of the incubator baskets are fitted with Nitex cloth (mesh size, 500μm). The cloth is secured by a removable, tightly fitting ring on the inside diameter of both the top and bottom pieces (Fig. 2). A retainer ring fixed to the outer wall of the incubator basket supports it in the water intake chamber. When an air stream is introduced into the base of the chimney, water is drawn through the intake chambers and incubator baskets into the base of the chimney and flows out the top. The chimney and water intake chambers are glued to an acrylic sheet base and acrylic sheeting is secured between the chimney and intake chambers to provide additional support. Current velocities are regulated by adjusting the air flow. The incubators must be deployed in water at least deep enough to cover the top of the chimney.

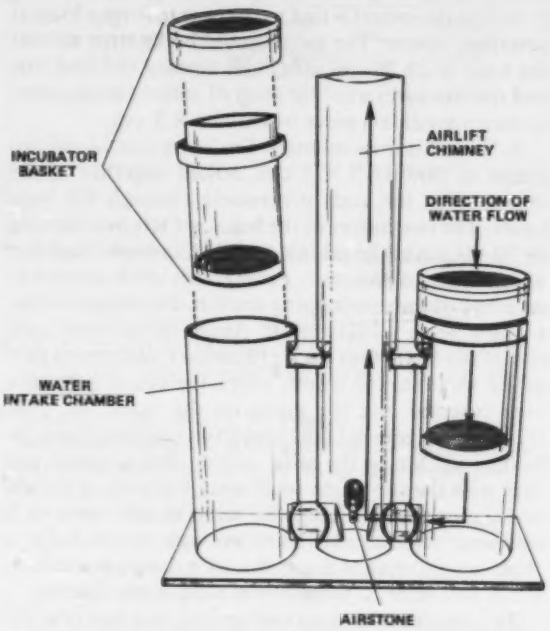

Fig. 1. Specifications for the airlift circulating incubator. Water intake chamber: 7 cm (OD), 3-mm sidewall, 15 cm high. Airlift chimney: 5.1 cm (OD), 3-mm sidewall, 30 cm high. A 2.5-cm (OD) port connects the chimney to the intake chambers, all of which are fixed to a base 24.0 X 9.5 cm and 6 mm thick.

The prototype of this incubator was constructed of polyvinyl chloride (PVC) pipe. If it is not necessary to monitor development visually, PVC may be more desirable than acrylic tubing, since it is easier to work with and is substantially less expensive. The incubators should be leached in water for not less than 3 days after the cement has cured, as a precaution against plasticizer toxicity.

Typically, hatching success of lingcod eggs incubated in this equipment was more than 90%. Several other species, including greenlings (*Hexagrammos* spp.), sailfin sculpin (*Nautichthys* spp.), cabezon (*Scorpaenichthys marmoratus*), tidepool sculpin (*Oligocottus maculosus*), and Pacific cod (*Gadus macrocepahalus*), have been hatched successfully in this incubator. This unit may also be suitable for incubating marine shrimp and crab eggs, as well as the adhesive

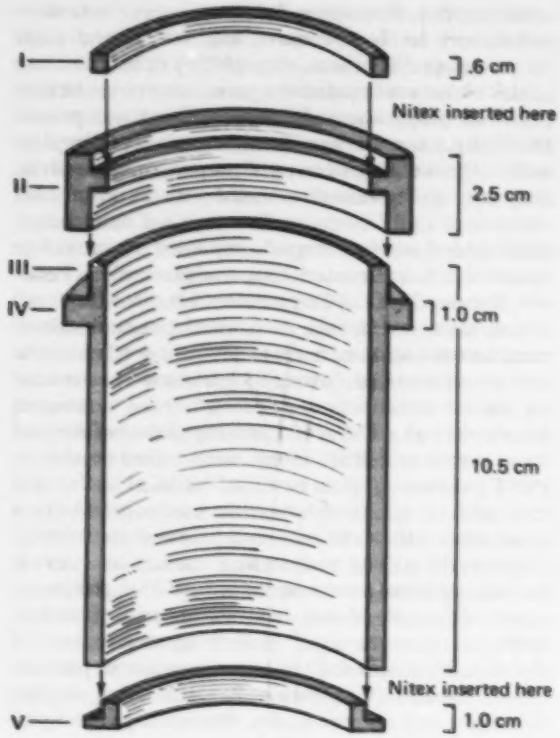

Fig. 2. Details of the incubator basket. Securement ring (I) for Nitex cloth: 6.5 cm (OD), 4-mm sidewall. Lid of incubator basket (II): 7 cm (OD), 6-cm sidewall, with the inside diameter milled to accept the securement ring and fit over the main body of the basket (III). Incubator basket (III): 6 cm (OD), 3-mm sidewall, 10.5 cm long. Retainer ring (IV): 7 cm (OD), 6-mm sidewall, with the ring milled to fit snuggly over the basket main body and cemented in place. Securement ring (V) for Nitex cloth bottom: 6 cm (OD), 6-mm sidewall, milled to fit securely into the bottom of the main body.

demersal eggs of freshwater fish species. The operational capability of this device in closed systems suggests it potential usefulness in toxicological investigations.

Acknowledgments

I thank Earl Prentice for his constructive suggestions and John Ellman and his staff for their outstanding craftsmanship in the construction of this device.

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Accepted 20 January 1982

Exposure of Largemouth Bass to Components of an Osmotic Infiltration Technique: Tolerance and Efficacy

When fishes are subjected to suboptimal environmental conditions, their natural resistance to disease may be significantly compromised. Bacterial epizootics in populations of susceptible fishes are common sequelae to stressful conditions. Treatment of bacterial disease in fishes may include removal or alteration of stressors and application of suitable antibacterial drugs. Although pathogens may be susceptible to various antibacterial drugs, the effectiveness of such drugs is transient and most have not been cleared for use in fish destined for human consumption.

Increased emphasis has recently been placed on the value of immunization as a preventive measure in the control of fish disease. Studies of the oral, parenteral, and infiltrative administration of antigens (vaccines) to fish have yielded inconsistent results. Oral immunization has not consistently produced high titers in young fish (Anderson and Nelson 1974; Klontz and Anderson

1970). Parenteral injection of a vaccine is not feasible when large numbers of small fish are to be immunized. Infiltration techniques are proven methods of effecting antigen presentation to fish (Amend and Fender 1976; Lannan 1978). We describe efforts to assess the tolerance of largemouth bass (*Micropterus salmoides*) to components of a hyperosmotic infiltration technique, and the efficacy of the technique.

Fingerling largemouth bass (3–5 cm) were obtained from a Federal fish-rearing station. Fish stocks were maintained at the Aquatic Animal Medicine Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, in fiberglass tanks capable of holding 1,400 L of dechlorinated tap water (pH, 6.8; Ca hardness, 5.0 mg/L). Water was filtered by recirculation through a 1:1 mixture of crushed oyster shell and charcoal. Experimental fish were maintained in a series of 4 90-L aquaria, connected in tandem to a filter containing the mixture of oyster shells and charcoal. Water temperature remained at 23 ± 2°C, and all fish were fed a commercial pelleted feed.

Fish were conditioned in combinations of solutes (sodium chloride, urea, magnesium sulfate) at various osmotic pressures (491–1,920 mOs) at ambient temperatures (22–23°C). Where applicable, osmotic pressures were determined by using freezing-point depression osmometry (Model 68-31LAS, Advanced Instruments, Newton Highlands, Massachusetts). Delivery (rehydrating) solutions were either dechlorinated tap water or a modified Ringer's solution: sodium chloride 0.6%, potassium chloride 0.006%, calcium chloride 0.012%, sodium bicarbonate 0.07%, in sufficient distilled water to equal 52 mOs (Guyton 1966:432).

Fish were placed and held in conditioning solutions for 3–10 min (using at least 500 mL aerated solution per fish), transferred to delivery solutions with nylon dip nets for a 6-min exposure, and then placed into several liters of aerated, dechlorinated tap water. After a 30-min equlibration period, fish were either bled or placed in experimental aquaria, depending on the experimental protocol.

In stage I experiments, fingerlings were subjected to a variety of conditioning solutions at ambient temperatures, followed by immersion in a delivery solution of dechlorinated tap water and removal to aquaria for observation. Deaths were counted 8 h after conditioning. A potential set of conditioning determinants was selected from these data.

In stage II trials, fingerlings and young adult bass were exposed to various conditioning solutions, then immersed in a delivery solution (modified Ringer's) containing 2.5 mg bovine serum albumin (BSA) per milliliter for 6 min. The maximum osmotic pressures employed in stage I conditioning (1,920 mOs) were lowered for stage II procedures to 1,694 mOs by using distilled water. Control fingerlings were immersed in the BSA/Ringer's solution for 6 min without prior conditioning. Following BSA exposure, fish were transferred to dechlorinated tap water, and after 30 min of equilibration, were bled by either puncture of the dorsal aorta (adults) or severence of the caudal peduncle (fingerlings). Fingerling blood was pooled (4–5 fish per sample) by collection into microtiter plate wells (Cooke Engineering Company, Alexandria, Virginia) which were then sealed with Parafilm "M" (American Can Company, Greenwich, Connecticut). Adult blood was transferred, unpooled, from syringe to sterile, silicone-coated stoppered glass tubes (Venoject, Terumo Medical Corporation, Elkton, Maryland) which were rested in a horizontal position. Blood samples were allowed to clot at 4°C for 4 h before the serum was removed. Serum BSA levels were estimated by use of rocket immunoelectrophoretic techniques (Axelson et al. 1973). Precipitating globulins derived from rabbit anti-BSA serum were added to molten (50°C) agarose (1% in modified barbital buffer, pH 8.6), and 15 mL of the mixture was poured onto a glass plate (10 × 10 cm) and allowed to solidify. Aliquots (5 μL) of test serum, normal bass serum containing known concentrations of BSA (reference standards), and normal bass serum were placed in wells cut into the agar. Following electrophoresis (8V/cm, 90 min at 4°C), plates were washed, pressed, dried, stained (Coomassie Brilliant Blue, 0.5%), destained, and allowed to dry. Precipitin peak heights were measured and recorded.

Stage I trials showed that combinations of solutes were less toxic to fingerlings than were single solutes, and that relatively high osmotic pressures could be tolerated for at least 3 min (Table 1).

Table 1. Stage I trials: Death of largemouth bass associated with osmotic conditioning in solutions of various concentrations of sodium chloride, urea, and magnesium sulfate.

Conditioning solution	pH	Time (min)	Number of fish	Mortality (No.)
NaCl 5%	5.5	3	5	5
NaCl 5%	7.0[a]	3	5	4
NaCl 2.5%	5.5	3	5	4
Urea 5%	6.0	3	5	0
$MgSO_4$ 5%	6.0	3	5	1
Urea 10.0%	6.0	3	10	1
Urea 10.0%	6.0	10	10	10
Urea 5% + NaCl 2.5%	7.0[a]	3	10	0
Urea 5% + NaCl 2.5%	7.0[a]	6	5	5
Urea 5% + NaCl 2.5% + $MgSO_4$ 2.5%	7.0[a]	3	10	0

[a] Adjusted to pH 7.0 with 1.0 *N* NaOH.

Rocket immunoelectrophoresis measurably detected BSA in infiltrated bass serum (stage II) at levels as low as 0.00015% (0.00075 μg/5-μL sample). Quantitation of BSA in sera following osmotic infiltration indicated that multiple-component conditioning solutions were more effective than single-component solutions (Table 2). The serum concentration of BSA following osmotic infiltration was found to be similar in fingerlings and young adult bass.

Results indicate that largemouth bass fingerlings tolerate hyperosmotic conditioning, and that hyperosmotic infiltration effectively accomplished the desired antigen presentation. Bovine serum albumin in osmotically infiltrated bass serum was easy to detect, whereas unconditioned fish exposed to BSA had no detectable BSA in their serum. In infiltrated fish, the correlation between serum BSA levels and BSA levels in other tissues (gills, lateral line, liver, muscle) is unknown. In addition, these findings may not necessarily be correlated with uptake of antigens other than BSA. Although no deleterious effects were attributed to the infiltrated BSA, other infiltrated antigens (particularly those of bacterial origin) may produce undesirable effects when fish are already compromised by osmotic stress. It may be necessary to establish optimal conditions for antigen administration for each antigen preparation to be used as a vaccine.

Table 2. Stage II trials: Rocket immunoelectrophoresis of bass serum following osmotic infiltration of BSA (F=fingerling; A=adult).

Sample	Number of pooled samples	Conditioning solution	Peak height	Serum BSA (10^{-2} μg/5μL)
F	10	Urea 5% + NaCl 2.5% + $MgSO_4$ 2.5%	7	2.0
F	10	Urea 5% + NaCl 2.5% + $MgSO_4$ 2.5%	6	1.5
F	5	Urea 5% + NaCl 2.5%	7	2.0
F	5	Urea 5% + NaCl 2.5%	6	1.5
F	10	Urea 10.0%	4	1.0
F	5	Urea 10.0%	3	0.75
F	10	No conditioning	0	0.00
A	2	Urea 5% + NaCl 2.5% + $MgSO_4$ 2.5%	7	2.0
A	1	Urea 5% + NaCl 2.5% + $MgSO_4$ 2.5%	5	1.25

Acknowledgments

This work was supported in part by an institutional SeaGrant program to Texas A&M University, NOAA Grant 04-8-M01-169, and the competitive grant program of the U.S. Department of Agriculture, SEA-CR-6421.

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Accepted 10 February 1982

First-year Growth and Survival of Bluegill and Black Bullhead Stocked with Largemouth Bass in South Dakota Ponds[1]

Largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) stocked together represent the most successful combination of pond fish species stocked in southern and midwestern regions of the United States (Regier 1962; Bennett 1970; Modde 1980). However, studies conducted in northern states such as Michigan (Ball and Tait 1952), New York (Regier 1963), and Montana (Brown and Thoreson 1952) have demonstrated that populations of bluegill and largemouth bass often are not successful.

The black bullhead (*Ictalurus melas*) has been stocked in ponds because it reproduces successfully and is easily caught by inexperienced anglers (Moorman 1957). However, the stocking of bullhead in ponds with bass has been questioned because of the tendency of bullhead to overpopulate. Due to their reproductive potential, black bullhead have not been recommended for stocking with bass in ponds in Montana (Brown and Thoreson 1951) or Indiana (Krumholz 1950). In contrast, Moorman (1957) stated that bullhead became overcrowded and stunted only when bass predation was insufficient to control excess recruitment. Rickett (1976) reported that excess bullhead recruitment was controlled in Kansas research ponds by stocking adult bullhead (1,250/ha) and adult bass (300/ha). The present study was conducted to evaluate the initial success of forage fish species stocked with largemouth bass in ponds of the northern Great Plains.

The study area consisted of the entire state of South Dakota. To enable both climatological and physiographical comparisons in pond fisheries, the state was divided into quadrants, with the east-west demarcation at the Missouri River and the north-south demarcation at latitude 42°21′.

Combinations of largemouth bass with bluegill and largemouth bass with black bullhead were stocked into four randomly selected ponds per quadrant, with the exception that eight ponds in the southeastern quadrant were stocked with bass and bluegill. Study ponds had a minimum depth of 3.5 m and ranged in area from 0.4 to 2.2 ha. The densities, stocking dates, and mean total length of the three species stocked in summer 1979 were 247/ha between 9 and 18 July for largemouth bass (37 mm), 988/ha between 24 and 28 July for black bullhead (34 mm), and 1,236/ha between 15 and 24 August for bluegill (22, 27, and 31 mm).

Fish were collected with an electroshocker, seines, and trap nets. Spring populations were estimated for black bullhead and bluegill in three ponds from the two eastern quadrants between 7 May and 11 June 1980.

The left pectoral spines of bullhead that were removed for growth analysis were sectioned at the distal end of the basal groove (Sneed 1951). Lengths of black bullhead at the first annulus were back-calculated by using the Dahl-Lee direct proportion equation. Acetate slide impressions of bluegill scales were examined on an Eberbach scale projector. Lengths of bluegill at the first annulus were back-calculated by using the corrected Lee equation. The body-lengths to scale-radius regression line equation for all bluegill scale samples collected was $L = 87 + 0.07\ S$. Because sample size per pond was small and there was little length variation, a standard value of 20.0 mm (K.D. Carlander, personal communication) was substituted for the y-intercept.

Mean condition (K) factors per bullhead (based on total lengths) were calculated for fish within four 15-mm length intervals during each of five 2-week periods from 1 May to 7 August 1980. Bluegill condition was evaluated by using the relative weight index, W_r (Anderson 1980).

Mean first-year growth was least for black bullhead in the two northern quadrants (Table 1). Duncan's multiple range test indicated a significant ($P < 0.05$) difference in first-year growth of fish in the northwestern quadrant and that of fish in the two southern quadrants, which can be attributed primarily to the differences in growing season. The growth rates observed (highest in the southeast, lowest in the northwest) coincide with the gradient in average

Table 1. Mean bluegill and black bullhead total lengths (in millimeters) at the first annulus for South Dakota quadrants (1979).

Species	Total lengths (mm) of fish in quadrants			
	NW	SW	NE	SE
Bluegill	58	52	50	48
Black bullhead	68	112	82	117

[1] Journal Series No. 1978 2 of the Agricultural Experiment Station, South Dakota State University.

number of growing days in South Dakota reported by Westin and Malo (1978).

The mean of 116 mm for first-year growth of black bullhead in southeastern South Dakota ponds was greater than the length reported for bullhead from established populations (Carlander 1969). The rapid growth rates observed in the present study were most likely due to low population densities and unexploited trophic resources. After one growing season, lengths of bullhead from ponds in southeastern South Dakota were equivalent to the minimum harvestable size (152 mm) for bullhead (Moorman 1957).

In the present study, mean total lengths of bluegill after the first growing season ranged from 48 mm in the southeastern quadrant to 58 mm in the northwestern quadrant (statewide average, 52 mm; Table 1). A one-way analysis of variance for mean first-year growth of bluegill along quadrants was not significant ($P > 0.05$). Carlander (1977) reported a decrease in bluegill growth rates at northern latitudes. The absence of latitudinal differences in growth of bluegill in South Dakota ponds during 1979 may have been due to the minimal number of growing days remaining after stocking (15–24 August 1979).

Black bullhead condition increased during May, sharply decreased between 29 May and 26 June 1980, and appeared to remain relatively stable from 26 June through 7 August (Fig. 1). Adjacent means per length-group were compared between sampling intervals by using a least significant difference mean comparison test. Results indicated a significant difference in condition ($P < 0.001$) within the four length-groups between 29 May and 26 June. Results also indicated a significant difference ($P < 0.001$) within length-groups 128.0–143.0 mm and 144.0–159.0 mm between early and late May.

The increase in black bullhead condition between 1 and 29 May and the decline between 29 May and 26 June may be attributed to differences in gonadal development for pre- and post-spawning periods. Bullhead spawning in South Dakota probably occurred between 29 May and 26 June. Boussu (1959) stated that the peak spawning period for black bullhead in southern South Dakota lakes apparently extends from the latter part of May through mid-June.

Average relative weight values per quadrant for bluegill ranged from 116 to 122 (statewide average, 120). A one-way analysis of variance for relative weight means among quadrants was not significant ($P > 0.05$). The analysis indicated no difference in mean relative weight for bluegill sampled statewide at different times during the second growing season. Although differences in relative weights of bluegill due to spawning were not detected, young-of-the-year bluegill were observed in at least two southeastern ponds. Age 1 bluegill spawning occurred in 100% of the Iowa study ponds (Hill 1977). Mean total lengths

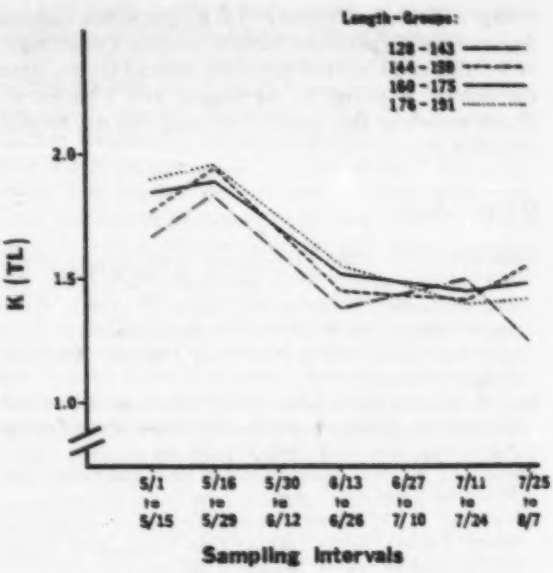

Fig. 1. Mean condition factors K (TL) for length groups (in millimeters) and sample intervals for black bullhead from South Dakota ponds (1980).

for age 1 bluegill in South Dakota ponds were similar to or greater than those reported by Hill (1977).

Bluegill survival in the present study, calculated for three eastern South Dakota ponds, ranged from 6 to 69% (mean, 32%). The mean survival calculated for eastern South Dakota ponds was comparable with survival reported from Iowa and Missouri. Hill (1980) observed a mean first-year bluegill survival between 26 and 32% for age 0 bluegill stocked (2,471/ha) with bass in five privately owned Iowa ponds. Novinger (1980) reported 44% survival of bluegill stocked (1,236/ha) simultaneously with bass in six Missouri research ponds between October 1974 and October 1975.

Black bullhead survival in the present study, estimated for three eastern South Dakota ponds, ranged from 62 to 71% (mean, 68%). This high first-year survival and the high reproductive potential of the species suggest that the stocking rate of 988/ha was too high. A stocking rate of 247/ha for bullhead, which is the rate recommended for channel catfish (*Ictalurus punctatus*) by most midwestern states (Modde 1980), might establish a harvestable population within one growing season with less tendency toward overpopulation.

Acknowledgments

This study was funded as a cooperative study by the South Dakota Department of Game, Fish and Parks

(Dingell-Johnson Project F-15-R), the South Dakota Agricultural Experiment Station, and the Department of Wildlife and Fisheries Sciences, South Dakota State University. Richard L. Applegate and Charles G. Scalet reviewed this manuscript and offered helpful criticism.

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Accepted 18 February 1982

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Aquarium Airline Tubing Marker for Observing Fish Movements

Randolph (1976) described and tested a marker for observing fish in shallow water areas in the natural environment that was made from rigid plastic tubing (3.18 or 1.59 mm in diameter) sealed at both ends. One end of the tubing was attached to the fish and the marker acted as a float that could be used to monitor movements. This marker was simple and inexpensive to construct, was retained by fish for long periods (8 months or more), and was tangle-resistant. Its use enabled identification of individual fish at a distance and had no apparent effect on behavior or mortality.

Markers of this type were used to monitor the movements of young sandbar sharks (*Carcharhinus plumbeus*) in an enclosure constructed in the natural

environment. The marker worked well for following general movements but was difficult to use for plotting the details of maneuvers made by a shark. Using Randolph's basic design, but substituting aquarium airline tubing for the rigid plastic tubing, I constructed a marker that had many of the same advantages and that provided more detailed information on fish movements.

The marker was made from a length of flexible aquarium airline tubing (4.76 mm in diameter) sealed at both ends with silicone sealer. A 20-cm piece of leather bootlace was inserted through a small hole made at one end of the tubing and used to tie the marker through a hole made at the posterior base of the first dorsal fin of the shark. Three size-13 corks were center-drilled, glued at 30-cm intervals to the opposite end of the tubing, and covered with reflective tape (Fig. 1). The corks increased the visibility of the marker and the tape enabled identification of individual fish and night observations with a spotlight. The cost of a marker 6 m long was about $0.65.

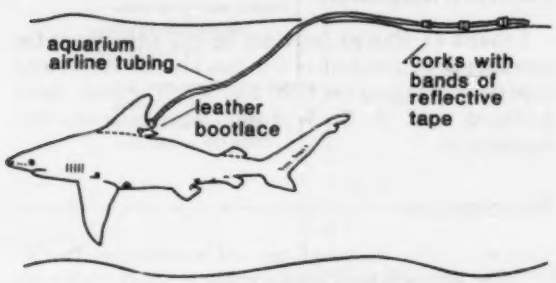

Fig. 1. Aquarium airline tubing marker for monitoring fish movements.

Unlike the rigid plastic marker used by Randolph, at least a 1-m portion of the flexible airline tubing marker could be kept on the surface of the water by making the length of the tubing several meters longer than the maximum water depth of the study area. The flexibility of the marker made it exceptionally responsive to movements and the top portion provided a detailed tracing, on the surface of the water, of the maneuvers made by a shark. Use of this marker made it possible to plot details of the movements of a shark during both day and night under a wide variety of experimental conditions and at distances of 100 m or more.

The marker has been tested on 15 sharks (total lengths 63–100 cm TL) kept for as long as 32 days in a salt marsh channel enclosure (110 × 75 × 2.5 m). A marker length of 6 m was sufficient to maintain at least a 1-m section on the surface of the water at all times. The marker did not become entangled with other markers or obstructions in the enclosure and did not twist into knots during periods of swimming with frequent changes of direction in small areas. Tagged sharks were not observed to chase or follow the marker. A marker pulled loose on only one occasion (when it was used to retrieve a shark). Each marker was in good condition when retrieved except for the accumulation of silt and algae on the reflective tape and probably would have remained attached for much longer periods of time. All sharks retrieved were in good condition and the proportion of stomachs containing food (9 of 14 examined) was similar to that reported for free-ranging sandbar sharks (Medved and Marshall 1981). Swimming speeds of marked sharks agreed with those obtained for sandbar sharks in the wild tagged with ultrasonic transmitters (Medved and Marshall 1983).

Although the airline tubing marker would probably not be as tangle-resistant as the rigid plastic marker in areas with dense vegetation or numerous obstacles, it would probably exert less drag on a fish in relatively open areas because of its flexibility.

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Accepted 21 January 1982

Retention of Coded Wire Tags in Lake Trout

Fingerling lake trout (*Salvelinus namaycush*) produced in Federal hatcheries and stocked to restore or supplement natural populations have been marked by fin clipping since the 1950's. However, the removal of certain fins can affect the survival of the fish and the number of possible fin-clip combinations is limited. Binary coded wire tags that are imbedded in the snout of salmonid fingerlings do not adversely affect the fish, and their use provides enough codes to mark several lots or year classes of fish (Hager and Jewell 1968; Ebel 1974). These wire tags have been used successfully in remote areas under field conditions (Koerner 1977).

Beginning in October 1979 and continuing through October 1981, personnel at the Allegheny National Fish Hatchery, Warren, Pennsylvania, have tagged 1,769,170 fingerling lake trout with the binary coded wire nose tags. During this marking effort, tag retention at the hatchery has been closely monitored. The present note documents information gathered from this marking program on short-term tag retention.

Trout were tagged with commercially available tagging machines (Northwest Marine Technology, Inc., Shaw Island, Washington). The machines were set up in the hatchery tank room where fish-holding space, water, and electricity were available.

The lake trout were anesthetized with MS-222, fin clipped, and tagged. The fin-clipping and tagging operation consisted of removing the adipose fin of an anesthetized fish, inverting it, and placing the snout of the fish in the head mold of the injector, where the tag was inserted by depressing a button. Tagged and untagged fish were separated as they passed through a quality control device. To ensure that tags had not been missed, rejected (untagged) fish were passed through the quality control device several times and then retagged. Tagged fish were transferred to rearing tanks or raceways at the hatchery.

The retention of coded wire tags in 193,490 fall fingerling lake trout of the 1979 year class (9.6–10.2 cm long) was 90% for 4,000 fish sampled 4 days after being tagged. In 509,315 yearling lake trout of the 1979 year class (14.4–15.2 cm long) tag retention was 96% for 4,000 fish sampled 95 to 182 days after tagging.

The relatively small size (9.6–10.2 cm, total length) of the fall fish from the 1979 year class, coupled with placement in the snout, caused problems with the proper placement of the tag and with tag retention. Both problems were overcome by redesigning the head mold. Adjustments that were made in the equipment included increasing the depth of the tag penetration into the snout and correcting the placement of the needle in the head mold.

Tag retention in 181,705 fall fingerling lake trout of the 1980 year class (10.6–11.1 cm long) held for 8 days after tagging was 97% for the 3,000 fish sampled. In 772,510 yearling lake trout of the 1980 year class (15.4–16.7 cm long) tag retention was 98% for 9,500 fish sampled 76 to 130 days after tagging.

Tag retention in 112,150 fall fingerling lake trout of the 1981 year class (11.1–11.8 cm long) held for 6 days after tagging was 98% for the 3,000 fish sampled.

Improvements in head mold design and tagging techniques over the 2 years of the program have resulted in a total increase of about 8% in retention of tags.

Acknowledgments

I thank G. Zalepa, who set up the equipment for marking large numbers of fish quickly; D. Blick, who supervised tagging the fish; and R. St. Pierre, John Leonard, and R. S. Wydoski, who reviewed the manuscript.

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Accepted 20 January 1980

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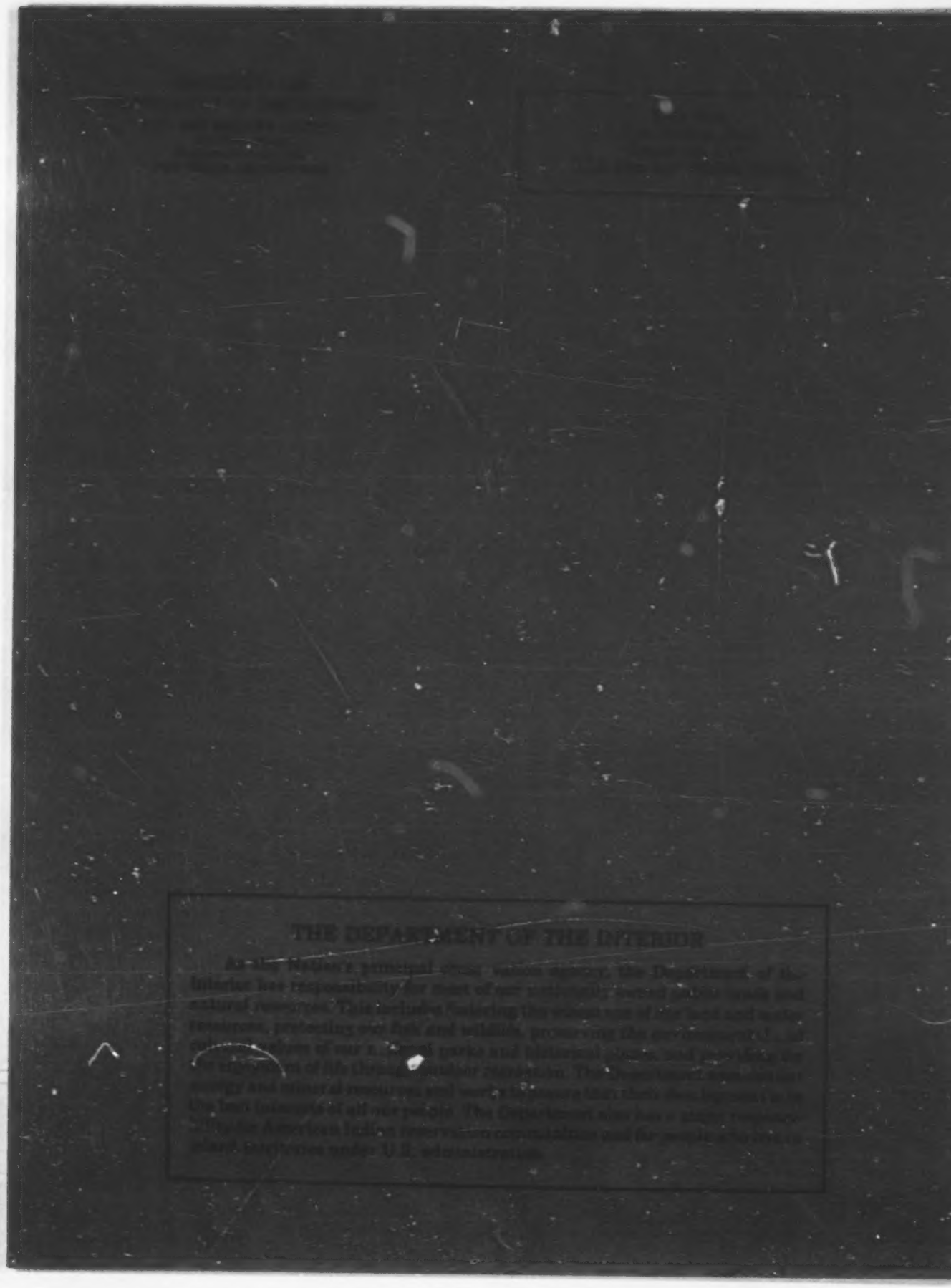

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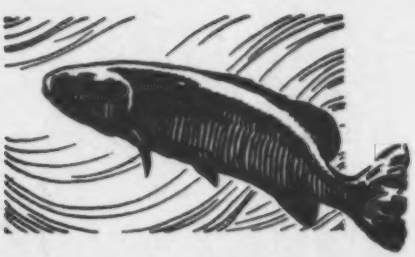

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Progressive Fish-Culturist

A Quarterly for Fishery Biologists and Fish-Culturists

Volume 44 October 1982 Number 4

CONTENTS

Immersion and Injection Vaccination of Salmonids Against Furunculosis with an Avirulent Strain of *Aeromonas Salmonicida*. *R.C. Cipriano* and *C.E. Starliper*.....167

Modification of Loudon Automatic Feeders. *D.W. Miller*.....169

Quality of Chum Salmon Fry Improved by Incubation over Artificial Substrates. *H.J. Fuss* and C. Johnson.....170

Food of Young Muskellunge in a Power Plant Cooling Reservoir. *R.J. Krska, Jr.* and *R.L. Applegate*.....172

Effect of Temperature on Survival of Northern Pike Embryos and Yolk-Sac-Larvae. *T.J. Hassler*.....174

Millimolar Quantities of Sodium Salts Used as Prophylaxis During Fish Hauling. *M.S. Haswell, G.J. Thorpe, L.E. Harris, T.C. Mandis,* and *R.E. Rauch*.....179

Transportation Techniques for Blueback Herring. *W.C. Guest* and *J.A. Prentice*.....183

Portable Barge for Applying Lime at Community Lakes. *W.C. Reeves*.....185

Factors Affecting the Efficiency of Clinoptilolite for Removing Ammonia from Water. *L.L. Marking* and *T.D. Bills*.....187

Fin Erosion and Culture-related Injuries of Chinook Salmon Raised in Floating Net Pens. *J.R. Moring*.....189

Evaluation of Fluorescent Pigment for Marking a Scaleless Fish, the Brook Stickleback. *G.E.E. Moodie* and *I.G. Salfert*.....192

Use of Self-feeders for Rearing Steelhead Trout at Dworshak National Fish Hatchery. *D.P. Statler*.....195

Toxicity and Residue Studies of Cultured Blue Shrimp Treated with the Algicide Cutrine-Plus. *R.R. Williams, J.E. Hose,* and *D.V. Lightner*.....196

Cost Analysis of Aquatic Weed Control: Fish Versus Chemicals in a Florida Lake. *J.V. Shireman*.....199

Induced Spawning and Culture of Bonytail Chub. *R.L. Hamman*.....201

Modified Procedure for Aging Walleyes by Using Dorsal Spine Sections. *T.L. Margenau*.....204

Plasma Corticosteroids and Chlorides in Striped Bass Exposed to Tricaine Methanesulfonate, Quinaldine, Etomidate, and Salt. *K.B. Davis, N.C. Parker,* and *M.A. Suttle*.....205

Effects of Tilapia Densities on Growth of Channel Catfish. *J.L. Wilson* and *L.R. Hilton*.....207

Studies on the Mineral Requirements of the Adult American Lobster. *M.L. Gallagher, R.C. Bayer, J.H. Rittenburg,* and *D.F. Leavitt*.....210

Spawning and Culture of Humpback Chub. *R.L. Hamman*.....213

Growth of Fed and Unfed Bighead Carp in Cages at Two Stocking Densities. *C.R. Engle*.....216

Infectious Hematopoietic Necrosis: A Comparison of Epizootics Among Fry Hatched from Saline-rinsed and Unrinsed Steelhead Eggs. *D.E. Ratliff, R.L. Hill,* and *W.J. Groberg, Jr.*.....217

Portable Trap for Collecting Adult Sea Lampreys. *R.J. Schuldt* and *J.W. Heinrich*.....220

Reviewers.....222

K. McLaren Redman

1919–1982

This issue of *The Progressive Fish-Culturist* is the last edited by K. Redman, who was killed in an automobile accident near Fort Collins on September 12. She had been the Editor since joining the staff of the Editorial Office of the Fish and Wildlife Service in January 1976. During the preceding 13 years she had advanced from editorial aide to Senior Editor at the National Center for Atmospheric Research, Boulder, Colorado. Awards for outstanding performance from both organizations attested to her successful pursuit of excellence. She came to the Service with a long-standing commitment to bring simplicity, lucidity, and good taste to science writing. And she did it tactfully, professionally, and extremely well. Aside from editing *The Progressive Fish-Culturist,* she often volunteered to assist in other work of the Editorial Office, and particularly relished applying her skills to long, complex, often loosely written manuscripts and molding them into clear, succinct, well-organized, and eminently readable reports. Invariably she gained the respect and appreciation of the authors, who readily recognized the superb quality of her work. She constantly strove to improve the content, style, and format of *The Progressive Fish-Culturist;* she made it a truly scholarly journal. You the readers and contributors will miss her expert touch, and we her colleagues will miss her extraordinary talent and inspiration.

Immersion and Injection Vaccination of Salmonids Against Furunculosis with an Avirulent Strain of *Aeromonas salmonicida*

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ABSTRACT: Replicate groups of brook trout *(Salvelinus fontinalis)* immunized by intraperitoneal injection of graded dilutions of an avirulent strain of *Aeromonas salmonicida* were protected from a virulent challenge. Mean mortality after challenge was 88% in control trout, 66% in trout immunized with 3×10^4 or 3×10^5 cells, and 39% in trout immunized with 3×10^6 to 3×10^7 cells. In brook trout challenged after immersion for 60 s in a culture containing 2.3×10^9 avirulent cells of *A. salmonicida* per milliliter, mortality was 44%, compared with 94% in controls. In Atlantic salmon *(Salmo salar)*, similarly vaccinated by immersion, mortality was 12.5% after challenge compared with 87.5% in controls. In additional studies, mean mortality of Atlantic salmon vaccinated by immersion was 14%, whereas that of control salmon was 92%.

Although Duff (1942) was the first to orally vaccinate trout against furunculosis, other attempts to mass-immunize fishes against *Aeromonas salmonicida* produced equivocal results (Paterson 1981). Protective antibodies are best produced against *A. salmonicida* by injecting fish with formalinized virulent cells of the bacterium emulsified in an adjuvant (Krantz et al. 1964; Paterson and Fryer 1974). Although a bacterin against furunculosis recently became commercially available, it requires delivery by injection. Therefore, a vaccine against furunculosis that can be delivered to fish by more practical means is still needed. The results of a recent study (Cipriano 1982) indicated that brook trout *(Salvelinus fontinalis)* can be effectively immunized by injection of extracellular antigens from either virulent or avirulent isolates of *A. salmonicida*. The purpose of the present study was twofold: (1) to determine if immersion in a suspension of avirulent cells of *A. salmonicida* was an effective alternative to parenteral administration, and (2) to compare the protection provided to brook trout so immunized with that of Atlantic salmon *(Salmo salar)*.

Materials and Methods

Two strains of *A. salmonicida* were used. One strain (3.95) was originally virulent, autoagglutinated in broth culture, and had an estimated LD50 of 138 cells per juvenile brook trout. After repeated agar passages and storage in broth culture for about 12 months, the strain became attenuated. It neither agglutinated in broth nor killed trout at concentrations 10,000 times greater than the original LD50 estimate. The second strain of *A. salmonicida* (3.101) was virulent for salmonids and was used to challenge test fish.

Hatchery-reared, specific-pathogen-free brook trout (34.7 g) and Atlantic salmon (28.6 g) were maintained in groups of 16 fish in 40-L aquaria. Each aquarium was supplied with a constant 1-L/min flow of 12.5°C spring water. Fish were fed a commercial trout ration three times daily.

A preliminary experiment was conducted to determine if attenuated strain 3.95 could immunize trout. The bacterium was cultured in broth and then diluted with physiologic saline in serial 10-fold steps to produce suspensions containing from 3×10^7 to 3×10^2 cells/mL. Concentrations were determined by dilution plate counts (Miles and Misra 1938). Subsamples of each dilution, 0.1 mL per fish, were injected intraperitoneally. Controls were injected with 0.1 mL of physiologic saline. No mortality occurred during 28 days of observation so the groups were then exposed for 60 s to a suspension of 1.2×10^8/mL virulent cells of strain 3.101. The daily mortality was recorded for 14 days after challenge. The specific cause of death was confirmed by the direct fluorescent antibody test (Bullock et al. 1980) and data were examined by chi-square analysis ($P < 0.05$).

Additional experiments were conducted to determine if immersion in suspensions of attenuated strain 3.95 would provide protection. Two groups each of brook trout and Atlantic salmon were immersed for 60 s in 2 L of broth culture containing about 2.3×10^9 living attenuated cells per milliliter. Controls were sham immersed for 60 s in sterile culture medium. Immersion in a suspension of the attenuated bacteria caused no mortality, and all groups were challenged with a suspension of virulent cells after 28 days. Because of their different sensitivity to the challenge, brook trout were exposed for 60 s to about 1×10^8 cells/mL and Atlantic salmon to about 1.8×10^7 cells/mL. Mortalities were recorded, the cause of each death was assessed, and data were analyzed as described for the injection experiment.

Before fish were immunized and again 2 weeks after challenge, 15 Atlantic salmon and 15 brook trout were bled. Mean humoral agglutinin titers were determined by the method of Maisse and Dorson (1976).

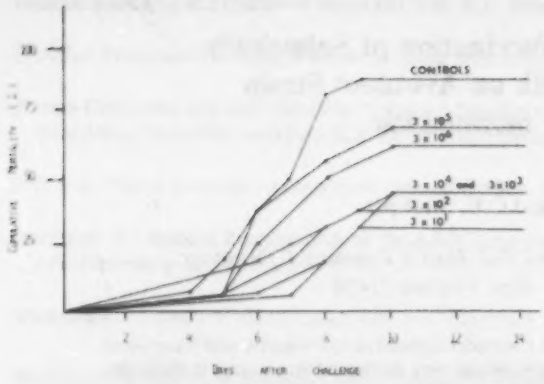

Fig. 1. Mortality among brook trout fingerlings immunized by intraperitoneal injection of six different concentrations (shown above each curve as number of cells per fish [3 × 10⁵ to 3 × 10¹]) of an attenuated strain of Aeromonas salmonicida *after exposure to 1.2 × 10⁸ cells of a virulent strain of the bacterium.*

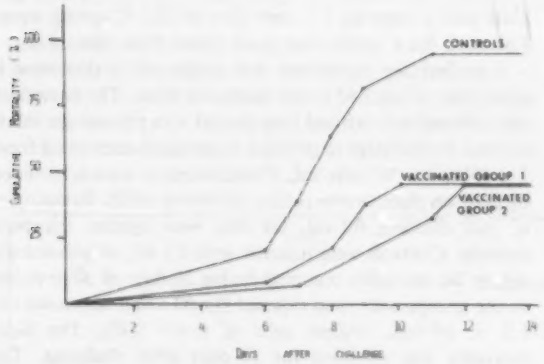

Fig. 2. Response of brook trout fingerlings immunized by immersion in 2.3 × 10⁸ cells of an attenuated strain of Aeromonas salmonicida *to exposure to 10⁸ virulent cells of* A. salmonicida.

To determine if the results were reproducible, four additional groups of Atlantic salmon were similarly vaccinated by immersion in the attenuated culture. Three additional sham-immersed groups of salmon served as controls. Test conditions were similar to those already described.

Results

Attenuated strain 3.95 evoked a protective immune response when administered parenterally to brook trout. Statistical analysis indicated that the protection observed among trout immunized with injections of either 3 × 10⁶ or 3 × 10⁵ cells differed ($P < 0.05$) from that observed among the other vaccinated groups. The mean mortality was 66% in trout immunized with 3 × 10⁶ or 3 × 10⁵ cells and 39% in other vaccinated groups; 87% of the controls died (Fig. 1).

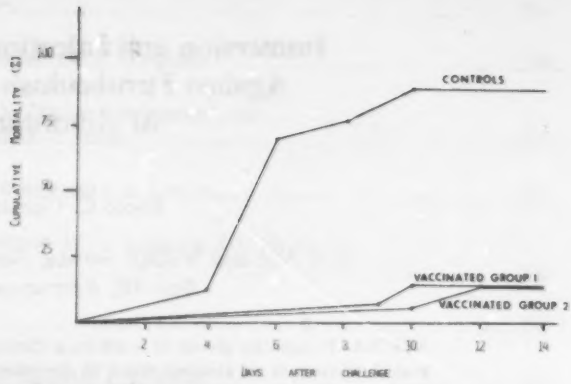

Fig. 3. Response of Atlantic salmon fingerlings immunized by immersion in 2.3 × 10⁸ attenuated cells of Aeromonas salmonicida *to exposure to 10⁷ virulent cells of* A. salmonicida.

Additional studies indicated that fishes were also immunized by immersion vaccination in the attenuated culture. Before immunization, brook trout and Atlantic salmon had mean titers of 3.8 and 4.4, respectively, against formalinized cells of *A. salmonicida*. In brook trout vaccinated by immersion and then challenged with 1 × 10⁸ cells/mL the mortality was 44% in each of two groups and the mean titer developed against the cellular antigen was 302.2 (Fig. 2). Among control trout the mortality was 94%; too few fish survived to permit calculation of a mean antibody titer.

Atlantic salmon similarly immunized by immersion in an attenuated culture of strain 3.95 had a 12.5% mortality in both vaccinated groups and developed a mean titer of 358.4 against the cellular antigen (Fig. 3). The mortality in control salmon was 87% within 14 days after exposure to 1.8 × 10⁷ cells/mL. Again, as in brook trout, not enough fish survived to support calculation of a mean antibody titer.

Additional results of immersion vaccination of Atlantic salmon in the attenuated culture showed that the observed protection was reproducible. Control salmon had a 92% mean mortality, whereas immersion-vaccinated salmon had a 14% mean mortality 14 days after a 60-s exposure to about 2 × 10⁷ virulent *A. salmonicida* cells (Fig.4).

Discussion

The attenuated strain of *A. salmonicida* did not harm fish and immersion in cultures of this strain protected trout and salmon against furunculosis. Immersing fish in a suspension of attenuated strain offers a practical and economic alternative to the injection of large numbers of small fish, but vaccination by this technique should be carefully considered before it is used in hatcheries where *A. salmonicida* is enzootic. Because the experimental challenge levels are substantially greater than those usually encountered in nature, further studies need to be performed on production-sized lots

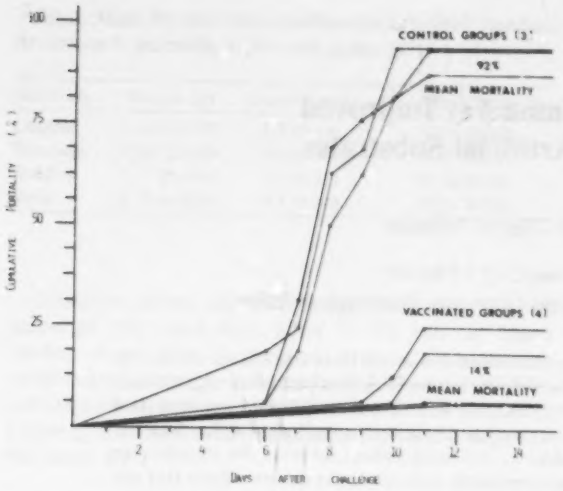

Fig. 4. Response of additional groups of Atlantic salmon fingerlings immunized by immersion in 1.6 × 10⁸ attenuated cells of Aeromonas salmonicida *to exposure to 10⁷ virulent cells of* A. salmonicida.

of fish to determine the actual field efficacy of the attenuated vaccine. The possibility of establishing carriers among immunized fish must also be assessed so that the transportation of fish from immunized lots will not violate current fish health policies.

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Accepted 14 April 1982

Modification of Loudon Automatic Feeders

The Loudon automatic feeders used by many fish cultural stations have a few drawbacks for efficient, trouble-free use. Over a short period of time, the screws used to hold the sliding troughs together tend to clog with feed. The clogging of these screw slots impedes operation of the unit.

A practice I put into use at Jordan River National Fish Hatchery is to cut the 6.5-cm foam rubber used for upholstery cushions into blocks 7 cm wide and 8 cm long and squeeze them in place on top of the screws and their related slots. These foam rubber blocks thus form a barrier to the fish food. During 6 months of continuous operation, no difficulties have been experienced with feeders equipped with these blocks.

Another use for foam rubber on these automatic feeders is to prevent moisture from getting to the feed. I squeeze a block of foam rubber 2.54 cm thick, 7 cm wide, and 1 m long into the trough over the feed to form a canopy. The heat tape supplied with the feeders, used in conjunction with the foam canopy, prevents excess moisture from entering the feed storage trough.

When the productin season ends, the blocks can easily be removed, washed, dried, and stored for use the next season.

— Dwayne W. Mills, *Jordan River National Fish Hatchery, Elmira, Mich. 49730.*

Accepted 2 April 1982

Quality of Chum Salmon Fry Improved by Incubation over Artificial Substrates

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ABSTRACT: Chum salmon (*Oncorhynchus keta*) fry were incubated over one of three artificial substrates and compared with a control group incubated over flat bare screen. Fry incubated over substrate were significantly larger in both mean weight and length ($P < 0.001$) than fry incubated without substrate. Fry size varied slightly among substrate groups but varied significantly due to tray position. Condition factors indicated that fry incubated over substrate completed yolk absorption sooner than fry incubated without substrate. We theorize that this is the result of lower levels of dissolved oxygen being immediately available to the alevins because they are less uniformly dispersed in the incubation container without substrate. Low levels of dissolved oxygen are known to retard yolk absorption in other salmonid alevins. Among the three substrate groups, Vexar was determined to be more time and cost effective than the other types of substrate tested.

Chum salmon (*Oncorhynchus keta*) are incubated in Heath-Techna incubators at some Washington Department of Fisheries (WDF) hatcheries. Hatchery personnel and biologists noted that the quality of fry produced in Heath incubators with the normal flat screen was poor. In a study of ways to improve fry quality, three types of artificial plastic substrate were compared with the control (flat screen) and data on the weight, length, and condition factors (K) of the fry were taken. The study took place at the George Adams Salmon Hatchery near Shelton, Washington.

Methods and Materials

Chum salmon eggs from a common egg take were randomly fertilized and randomly placed in four stacks of Heath-Techna incubators. Three stacks (16 trays per stack) were randomly assigned to each of three experimental treatments: 1.9-cm polyvinyl chloride (PVC) Vexar, 2.5-cm PVC Intalox saddles, or 3.8-cm PVC Bio-rings. A fourth stack was designated as the control and consisted of fiberglass screen trays (14 mesh/cm). The uppermost tray in each stack was not used. Trays in the experimental groups were filled with substrate to a depth of 3.8 cm and loaded at a density of 7.05 alevins/cm² (Table 1). The incubators were supplied with well water at 10°C and 19 L/min flow. Water hardness, pH, and dissolved oxygen levels were all normal for salmonid incubation. After 88 days (880 temperature units, Celsuis degrees) 50 fry from each of four trays (two each from the top and bottom) per stack were weighed (to the nearest decigram) and measured (to the nearest millimeter). The hypothesis of equal fry weights in the four groups was tested by using one-way analysis of variance. In addition, positional differences within each stack were tested by using one-way analysis of variance and the Student-Newman-Keuls procedure for differentiating non-homogenous groups. Two-way analysis of variance was initially used, but because of a statistically significant two-way interaction effect of substrate and tray position on weight, we decided that more information could be gained by doing two one-way analyses.

Table 1. Alevin densities for each substrate.

Substrate	Substrate size (cm)	Alevin density		Adjusted alevin density[a]	
		No./cm²	No./cm³	No./cm²	No./cm³
Control	Flat screen	7.05	1.39	7.05	1.51
Bio-rings	3.81	7.05	1.39	7.67	1.51
Saddles	3.81	7.05	1.39	7.58	1.51
Vexar	1.91	7.05	1.39	7.58	1.49

[a] Density adjusted by calculating void space of substrate: Bio-rings and saddles, 92%; Vexar, 93%.

Results

Fry incubated over any of the three substrates were significantly larger in both mean weight and length ($P < 0.001$) than fry incubated without substrate (Table 2). Additionally, fry incubated with Bio-rings were somewhat larger than fry incubated with Vexar or saddles; this difference, however, amounted to only 0.1 g and is probably not biologically significant. Mean fry size also varied with tray position (Fig. 1). There were significant positional differences in mean weight in the Vexar, Bio-ring, and control groups, but the mean differences in weight did not exceed 0.03 g and are probably not biologically significant.

Table 2. Mean fry sizes and condition factors (with standard deviations in parenthesis) for each substrate.

Substrate	Weight (g)	Length (mm)	Condition factor (K)
Control	0.31 (0.038)	3.5 (0.14)	0.74 (0.02)
Bio-rings	0.38 (0.036)	3.8 (0.13)	0.67 (0.02)
Saddles	0.37 (0.035)	3.8 (0.13)	0.72 (0.04)
Vexar	0.37 (0.035)	3.8 (0.14)	0.71 (0.02)

Condition factors (K) were computed to determine the degree of yolk absorption. Since the fry had not begun feeding, the condition factor would be expected to continue to fall below unity as the yolk is absorbed. The mean K of the substrate groups combined was lower than that of the control (Table 2). Among the substrate groups, the Vexar groups had the lowest K.

Discussion

The use of artificial substrate produced fry of greater weight than bare screen substrate. The difference among the three substrates was minimal and probably not biologically significant. Shroder (1976) found that fry incubated on gravel or astroturf were significantly larger than those incubated on bare screen. When subjected to a controlled feeding regime for 5 weeks, the fry incubated on either of the two substrates were still larger than fry incubated on bare screen. However, the actual growth rates of the fry incubated over substrate and over bare screen did not appear to be different. Leon (1975) found that fry of Atlantic salmon (*Salmo salar*) incubated on plastic substrate were 45% larger than control fish. The substrate-incubated fry maintained a size advantage for 225 days after hatching. No controlled feeding trials were done in the present experiment. However, since the fry were reared for 65 days before release, it is possible, based on Shroder's and Leon's findings, that the fry incubated on substrate were larger at release than the fry incubated on flat screen.

Although the difference in the size of fry from the three substrate groups was minimal, there appear to be other benefits from the use of substrate rather than bare screen in Heath incubators. For instance, the substrate groups complete yolk absorption sooner than the control groups and, therefore, begin feeding sooner than the control group fish; this was noted by Leon (1975) and has also been observed by the authors with chinook salmon (*O. tshawytscha*). Also, fry dispersion at ponding appears to be more uniform in substrate-incubated fry. Chinook salmon fry incubated over bare screen tended to congregate in large masses on the bottom of the pond (personal observation). Leon (1975) also noted this type of behavior with Atlantic salmon fry incubated without .ugose substrate. We believe this dispersal has substantial benefits in reducing yolk sac abrasion and subsequent problems (coagulated yolk and malformed yolk sac caused by abrasion of the yolk sacs on the cement). It was not

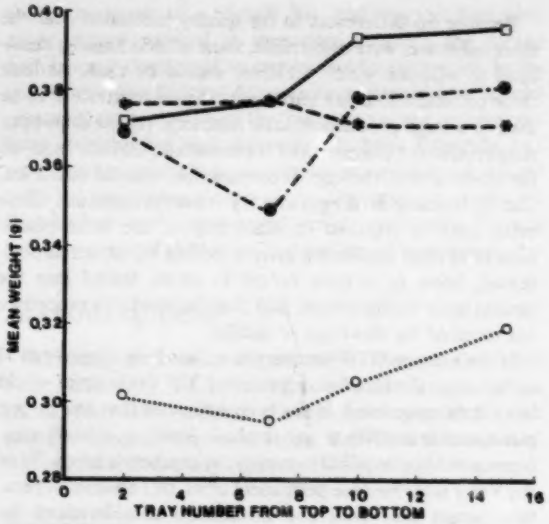

Fig. 1. Mean weight of chum salmon alevins from top to bottom of Heath incubator stack. Open circles = controls, bullets = Vexar, squares = Bio-rings, and asterisks = saddles.

determined why fish incubated over substrate completed yolk absorption sooner than fish incubated over bare screen. Generally, it is believed that incorporating substrate during incubation reduces alevin movement, thus allowing more energy to go into tissue growth than into maintaining position. Brannon (1965) showed that sockeye salmon (*O. nerka*) incubated at low oxygen concentrations took 2 weeks longer to reach complete yolk absorption than did fry incubated at high oxygen levels. We have observed a more uniform alevin distribution in numerous types of incubators that contained substrate. Conversely, fry incubated without substrate often clump together, sometimes to the point of smothering themselves. It is possible that this clumping behavior reduces the environmental levels of oxygen immediately available to the alevins, thus retarding yolk absorption to some degree.

An additional potential advantage of using artificial substrate is reduction of certain diseases. Unpublished WDF data indicated that the mortality of chinook salmon due to coagulated yolk disease was nearly 10 times greater in fish incubated without substrate than in substrate-incubated fish. Also, mortality due to cold-temperature disease (*Cytophaga psychrophillia*) was statistically higher for coho salmon fry (*O. kisutch*) incubated without substrate than for fry incubated with substrate. The incidence of constricted yolk sacs was much higher in Atlantic salmon fry incubated without substrate than with substrate (Leon 1975). Although no quantitative data relating to disease incidence were taken in the present study, the incidence of coagulated yolk and constricted yolk sacs appeared to be less in the substrate-incubated groups.

Because no differences in fry quality incubated over the three substrates were discernible, some criteria must be developed to evaluate which substrate should be used. At least three considerations are important when a substrate is to be used in a large production-scale hatchery: (1) the man-hour requirement for placement of the substrate, (2) time required for working with the eggs in conjunction with the substrate, and (3) cleaning dead eggs and fry from the substrate. Little extra labor is required to place any of the three plastic substrates in an incubation system. Before Vexar can be positioned, however, it must be cut in strips, folded into the desired layer configuration, and then fastened — a procedure not required for Bio-rings or saddles.

If the average WDF hatchery has a hatching capacity of 11 million eggs (in Heath incubators), 1,375 Vexar units would have to be assembled. It has been estimated that one person can assemble one Vexar unit in about 3 min, so that 69 man-hours would be required to supply the hatchery's needs. Since the Vexar units become permanent units, this assembly operation occurs only once and installation considerations for Vexar are similar to those for the other types of substrate.

The most important consideration may be the man-hour requirements for egg picking and for cleanup after the fry have been placed in ponds. In large production facilities, eggs are shocked and picked en masse. The substrate must be in the incubator when the eggs are replaced for subsequent incubation. Both the Bio-rings and saddles are more labor intensive than Vexar because they cannot be handled as a single unit and they are more difficult to clean of dead eggs and fungus. The substrate must also be cleaned and stored after the fry have been placed in ponds. At this stage the Bio-rings are more labor intensive than the saddles or Vexar because dead eggs and fry and associated fungus tend to form mats in the interstices. Because these mats are difficult to remove with high-pressure water jets, the individual pieces must be washed en masse. Large quantities of either Bio-rings or saddles can be soaked in a solution of LLMO (Live Liquid Micro Organisms), which satisfactorily removes the mats but does not dissolve unbroken eggs (D. Popochock, personal communication), and additional cleaning is therefore necessary. Cleaning Vexar is much easier; shaking dead eggs and fry from the substrate and then hosing each unit off is all that is usually required. A cost comparison is given in Table 3.

Table 3. Cost comparison of the three substrates, based on the use of 1375 Heath trays.

Substrate	Handling and installation		Cleanup		Total	
	Hours	Cost ($)	Hours	Cost ($)	Hours	Cost ($)
Bio-rings	17	97	27	153	44	250
Saddles	17	97	27	153	44	250
Vexar[a]	5	28	17	97	22	125

[a] Does not include preliminary assembly time.

Larger fry are produced when an artificial plastic substrate is used with Heath-Techna vertical incubators. The increased size of the fry produced with substrate is probably the result of more efficient yolk assimilation because energy is being used for tissue growth rather than for swimming and maintaining position. No discernible differences in fry size resulted from the use of different types of substrate, indicating that all three provided similar benefits to the alevins. The use of Vexar substrate is less time-consuming and more cost efficient (after initial assembly) than either Bio-rings or saddles.

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Accepted 26 April 1982

Food of Young Muskellunge in a Power Plant Cooling Reservoir

Fingerling muskellunge (*Esox masquinongy*), 36-280 mm in total length, were stocked in 1978 and 1979 in the cooling reservoir of the Big Stone Power Plant in northeastern South Dakota. At normal water level of 341 m above mean sea level, the reservoir has a surface area of 145 ha, maximum depth of 10 m, and mean depth of 4.9 m. Among the 18 species of forage fish in the reservoir when muskellunge were introduced, 4 predominated: yearling and 2-year-old bluegills (*Lepomis macrochirus*), tadpole madtoms (*Noturus gyrinus*), orangespotted sunfish (*L. humilis*), and black bullheads

Table 1. Stomach contents of 107 muskellunge (60-386 mm total length), expressed as percentage volume and percentage of total number of organisms. Fish were collected from a power plant cooling reservoir in South Dakota, August 1978–January 1980.

Organism	Percent of Volume	Percent of Number
Mayfly nymph	0.2	10.9
Johnny darter	23.8	30.9
Fathead minnow	24.1	5.5
Bluegill	35.1	14.5
Orangespotted sunfish	7.6	9.1
Unidentified *Lepomis* spp.	1.5	9.1
Unidentified fish remains	7.7	20.0

(*Ictalurus melas*). The estimated standing crop of these four species combined was 28 kg/ha (Wahl and Applegate, in press). The mean lengths of the stocked muskellunge at ages I and II considerably exceeded those reported for muskellunge in other waters in North America (Wahl and Applegate 1981).

All muskellunge that were impinged on two vertical traveling screens at the power plant intake were collected each day from August 1978 to January 1980 and preserved in 10% formalin. The fish were measured to the nearest millimeter (total length), and stomachs (from the esophagus to the pylorus) were removed. Organisms eaten by the fish were identified to species or genus and measured to the nearest millimeter (total length), and their volumes were determined by water displacement.

Of 107 muskellunge (60-386 mm long) collected, the stomachs of 47 contained food. The food consisted primarily (Table 1) of bluegills, orangespotted sunfish, johnny darters (*Etheostoma nigrum*), and fathead minnows (*Pimephales promelas*). The length ranges of prey fishes for which accurate measurements could be obtained were 17-62 mm for *Lepomis* spp. and 31-58 mm for johnny darters and fathead minnows. Expressed as a percent of muskellunge length, the ranges were 17-31% and 26-37%, respectively. Tiger muskellunge (*Esox masquinongy* × *E. lucius*), 90-170 mm long, selected bluegills and minnows of similar relative length range (Gillen et al. 1981).

Oehmcke et al. (1958) stated that adult muskellunge prefer soft-rayed forage fishes. Hourston (1952) reported that fishes with a cylindrical shape dominated the diets of adult muskellunge in Canada, but according to Crossman (1962), this may have been due to selection by size and not by species. Buss (1960) also stated that muskellunge prey on whatever fishes are available of the preferred size. Gillen et al. (1981) found that pellet-reared tiger muskellunge had only a limited ability to convert to a bluegill diet, and that this limitation might reduce survival in reservoirs in which the forage population is dominated by centrarchid forage species. In the present study, cylindrical fishes (johnny darters and fathead minnows) made up 48% of the food volume, but the predominant deep-bodied and spiney-rayed fishes (bluegills and orangespotted sunfish) also contributed a substantial volume (44%). Thus young-of-the-year muskellunge appear to eat deep-bodied and spiney-rayed fishes when they form a prominent part of the forage base and are of appropriate size.

Acknowledgments

We thank R.G. Johnson and N.N. Thomas, Otter Tail Power Company, for providing access and facilities at the cooling reservoir. Funding for this study was provided in part through Dingell-Johnson Project F-15-R and the South Dakota State Universtiy Agricultural Experiment Station.

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Accepted 18 February 1982

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Effect of Temperature on Survival of Northern Pike Embryos and Yolk-sac Larvae

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ABSTRACT: Experiments were conducted to examine the effect of water temperature and handling procedure on the percentage hatch of known-age northern pike (*Esox lucius*) embryos and on survival of the yolk-sac larvae. Embryos and larvae were incubated at constant temperatures from 3 to 24 °C. The hatching temperature range was 3.1 to 20.9 °C and lethal temperatures were 3 and 24 °C. Optimum hatching range was 6.2 to 20.9 °C and maximum hatch occurred at 9 to 15 °C. The age of the embryo before temperature change and the test temperatures both had a significant effect on percentage hatch. Temperature had the greatest adverse effect on percentage hatch during the first 5 h of development. There was no significant difference in the number of embryos hatching when comparing those that developed for 5 h before temperature change with those that developed 12, 24, and 48 h before change. There was a significant increase in hatch for embryos that had developed 120 h before change. The type of temperature change (shock or tempered) did not significantly influence the percentage hatch or hatching temperature range of embryos 5 h old or older. Management implications are also discussed.

The northern pike (*Esox lucius*), an important sport fish in Lake Oahe and Lake Sharpe, two mainstem Missouri River reservoirs in South Dakota, has fluctuated markedly in year-class strength (Elrod and Hassler 1969; Hassler 1969, 1970). These fluctuations, in part, have been associated with prolonged low water temperatures during spawning. Incubation of embryos in the reservoir indicated a minimum temperature tolerance of about 5 °C.

Reported laboratory studies have dealt with the effect of water temperature on hatching success and larval survival of northern pike. European studies by Volodin (1960), Swift (1965), and Lillelund (1966) demonstrated that hatching of northern pike embryos occurred over a temperature range of 3.7 to 21 °C. Lillelund found that the least developed embryos showed the highest sensitivity to increased temperature and that larvae were more tolerant of elevated temperatures than were embryos. Hokanson et al. (1973) developed bioassay methods for determining the temperature requirements of 15-min and 5.5-h-old embryos and 1-day-old and first-swimming yolk-sac larvae.

The present study was undertaken as a followup to studies on hatching success and survival of larvae under natural conditions (Hassler 1970). The objectives of the present study were to determine the effect of water temperature and handling technique on (1) percentage hatch of 2- to 192-h-old northern pike embryos acclimated to or placed immediately in test temperatures and (2) the survival of 1-h-old yolk-sac larvae.

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Methods

Apparatus

A 378-L refrigerated, recirculating, insulated fiberglass tank was used at the three lowest temperatures for rearing. At other temperatures a 46-L flow-through Styrofoam tank having an average flow of about 75 L/day was used. In the Styrofoam tanks, temperature was controlled by thermostatic regulation of immersion heaters equipped with a stirrer. Fertilized eggs were held in floating, nylon-screen holding trays in the tanks before being transferred to pint jars or plastic cylinders 6 cm in diameter covered with nylon screen. The eggs did not roll during incubation. Larvae were reared in the screen-covered jars. The tanks were equipped with racks which held the jars and cylinders near mid-depth. All experiments were conducted in constant incandescent light.

Temperature in the fiberglass tanks was controlled within a range of 1 Celsius degree: mean recorded temperature at 3 °C was 3.1 ± 0.3 °C; at 6 °C, the mean was 6.2 ± 0.2 °C; and at 9 °C, the mean was 9.2 ± 0.3 °C. Temperature control in the Styrofoam tanks was within a range of 0.5 ± 0.1 Celsius degree. Temperatures were recorded daily with a mercury thermometer and monitored with recording thermographs. Water supply was aerated raw well water.

Procedure

Four experiments were conducted to determine the effect of six handling procedures and eight constant temperatures on the percentage hatch of known-age northern pike embryos. The temperature at which 50% of the embryos survived to hatch, median tolerance limit (TL50), was deter-

Table 1. Data on source of fish and handling procedures.

Year	Capture area	Females fertilized (no.)	Fert. temp. (°C)	Hours eggs held at fert. temp. before tempering	Mean no. of eggs per container
1969	Lake Sharpe	8	10	36	95/jar
1970	Lake Sharpe	4	9	2	120/jar
1971	Lake Oahe	2	12	5[a] and 24	50/cylinder

[a]One group of eggs was transferred directly to test temperatures after 5 h (shocked).

mined by using normalized percentage values, the highest hatch adjusted to 100%. The normalized results were plotted against an arithmetic temperature scale as described by Hokanson et al. (1973).

Ripe northern pike were captured with trap nets on the spawning grounds where eggs were stripped and fertilized by the dry method (Sorenson et al. 1966). Data on handling procedures are listed in Table 1. Fertilized eggs were transported to the laboratory in a Styrofoam carrying case and transferred to holding trays in the temperature control units set at the temperature of fertilization. Spawning and transport took 1 to 3 h.

After tempering, randomly selected living embryos were transferred from the holding tray to the containers. The jars and cylinders were placed inverted on the rack in the temperature control units and incubated at constant test temperatures. During incubation, after the third day, one container from each test temperature was removed daily and the embryos were preserved in 5% buffered formalin. Preserved samples were examined with a dissecting microscope to determine the percentage hatch (the ratio of the number of embryos that survived to hatch to the total number of embryos per container). Dead and deformed yolk-sac larvae were excluded from the hatch.

In 1971 the percentage hatch was compared in two experiments. In one, the hatch at test temperatures of shocked embryos 5 h old was compared with that of tempered embryos 5 and 24 h old, and the hatch of tempered embryos 5 h old was compared with that of tempered embryos 24 h old. In the second, a transfer study, embryos 5 h old were tempered to holding temperatures of 6 to 18 °C and held for 0.25, 0.5, 1, 2, 4, 5, 7, and 8 days. After the various tempering periods, randomly selected living embryos were transferred from the trays at each holding temperature to cylinders and then moved directly to test temperatures. Control groups were subjected to the same amount of handling. The hatch of embryos transferred from holding to test temperatures was compared over time.

Two experiments were conducted to determine the effect of temperature and handling procedure on yolk-sac larvae. One hour after hatching 25 yolk-sac larvae from each hatching temperature (3 to 21 °C) were placed in jars; one jar was returned to its respective hatching temperature and one was transferred directly to each test temperature. Survival of the unfed yolk-sac larvae was observed for up to 26 days.

The fertilized eggs were tempered at 3 °C/h from fertilization to test temperatures of 3 to 24 °C, graduated at intervals of 3 °C. The eggs were pooled each year.

Data were analyzed by using Friedman's rank test, Wilcoxon's signed rank test, and Spearman's correlation test (Langley 1971). The level of significance is accepted as being $P < 0.05$ or 0.01.

Results

Embryo survival

Embryos hatched over a temperature range of 3.1 to 20.9 °C and lethal temperatures of 3 and 24 °C were the same

Table 2. Percentage normal hatch of northern pike embryos subjected to five handling procedures and held at eight temperatures. Normalized hatch (highest hatch adjusted to 100) is given in parentheses.

Mean water temperature (°C)[a]	Hours developed, experiment, and procedure				
	Experiment 1[b]	Experiment 2[c,d]	Experiment 3[e]		
	36 tempered	2 tempered	5 shocked	5 tempered	24 tempered
3.1	30 (34)	10 (16)	26 (32)	24 (28)	23 (26)
6.2	83 (93)	53 (85)	75 (91)	80 (93)	73 (84)
9.2	—[f]	52 (84)	82 (100)	81 (94)	75 (86)
11.7	89 (100)	62 (100)	76 (93)	86 (100)	87 (100)
15.2	80 (90)	39 (63)	76 (93)	70 (81)	87 (100)
17.9	75 (84)	29 (47)	64 (78)	71 (82)	73 (84)
20.9	79 (89)	21 (34)	58 (71)	65 (75)	74 (85)
24.2	—	0	0	0	0

[a]Water temperature had a significant effect on normalized hatch in all experiments ($P < 0.05$, Friedman's test).
[b]Developed 36 h at fertilization temperature (10 °C) before being tempered to test temperatures.
[c]Developed 2 h at fertilization temperature (9 °C) before being tempered to test temperatures.
[d]Normalized hatch was significantly lower in experiment 2 than in experiments 1 and 3 ($P < 0.05$, Friedman's test).
[e]Developed 5 and 24 h at fertilization temperature (12 °C) before being tempered or directly transferred to test temperatures.
[f]Unit malfunction.

Table 3. Effects of temperature on northern pike embryo survival to hatch for embryos tempered at 3°C/h from fertilization to test temperature.

Time eggs developed before temperature change (h)	TL50 (°C)		Hatching range (°C)	Optimum hatching range (°C)	Maximum hatch	
	Lower	Upper			Temperature (°C)	%[a]
2	4.5	17.5	3.1-20.9	6.2-15.2	12	62
5[b]	3.9	21.9	3.1-20.9	6.2-20.9	9	82
5	4.0	22.0	3.1-20.9	6.2-20.9	12	86
24	4.1	22.2	3.1-20.9	6.2-20.9	12, 15	87
36	3.8	—[c]	3.1-20.9	6.2-20.9	12	89

[a]Significant correlation between embryo age before temperature change and maximum hatch ($P < 0.05$, Spearman's correlation test).
[b]Transferred directly from fertilization temperature to test temperatures (shocked).
[c]Survival was more than 50% at the highest test temperature (20.9°C).

for all experiments (Tables 2, 3). Water temperature had a significant effect on normalized hatch in all experiments (Table 2). Hatch was highest at 9 to 15°C and lower at the other test temperatures. Normalized hatch was significantly lower in experiment 2 than in experiments 1 and 3 (Table 2). There was no significant difference in hatch between experiments 1 and 3. There was a significant correlation between embryo age before temperature change and the percentage maximum hatch (Table 3). Maximum hatch increased as age before temperature change increased. However, there was no significant correlation between embryo age before temperature change and the lower TL50.

In experiment 3, water temperature had a significant effect on hatch for all treatments (Table 2). There was no significant difference in hatch between treatments. The optimum temperature range for batching was 6.2 to 20.9°C for all treatments.

Transfer Experiment

Water temperature had a significant effect on hatch of embryos transferred from 6 and 15°C, but age at transfer did not (Table 4). The hatch was lowest at 3°C and highest at 6 and 15°C. At 9 and 12°C both test temperature and embryo age before transfer had a significant effect on hatch. The hatch was lowest at 3°C and maximum at 6 to 15°C. The hatch was significantly higher for embryos incubated for 7 days at 9°C than for embryos incubated for 0.5 to 5 days before transfer. Embryos incubated at 12°C for 5 days had significantly higher hatches than those incubated for 6 h to 2 days before transfer (Table 4). Embryos incubated at 18°C for 12 h had significantly higher hatches than those incubated for 6 h before transfer (Table 4). The hatch was lowest at 3 and 6°C.

In one experiment embryos were fertilized at 10°C and tempered to 3°C and held for 6 days at that temperature before direct transfer to test temperatures. Survival to hatch was:

Test temperature (°C)	Percentage Hatch
12	70
15	75
18	70
21	20

In most experiments at temperatures above 15°C there was some egg fungus infection, which could have increased mortality. However, when samples were examined it appeared that the fungus was confined to dead eggs.

Survival of Yolk-sac Larvae

None of the yolk-sac larvae that hatched at, or were transferred to, 3°C survived to the free-swimming stage. Survival was 90% or more at other hatching temperatures (Table 5). Yolk-sac larvae transferred from 3°C survived only at 6°C. Those transferred from 6°C did not survive at temperatures above 15°C. Survival was 44% or more for all other transfers (Table 5). The lower 10-day TL50 for yolk-sac larvae held at hatching temperature was 4.2°C. Survival was more than 50% at higher temperatures.

Both hatching temperatures and test temperatures had a significant effect on survival of yolk-sac larvae (Table 5). Survival at test temperatures was significantly higher for yolk-sac larvae transferred from hatching temperatures of 9 to 21°C than for those transferred from 3 and 6°C. Survival of yolk-sac larvae was significantly lower at test temperatures of 3 and 21°C for all comparisons.

Survival and Temperature Shock

Embryos survived temperature reductions of 15°C and increases of 18°C, and hatched normally (Table 4). Mean hatch of embryos transferred directly from 18 to 3°C was 14% and for direct transfer from 3 to 21°C, 20%. Yolk-sac larvae survived temperature reductions of 15°C and increases of 12°C, and developed to the free-swimming stage (Table 5). Survival was 68% when transfer was direct from 21 to 6°C and 72% for direct transfer from 9 to 21°C. Embryos

Table 4. Percentage normal hatch of northern pike embryos transferred directly to test temperatures after incubating at holding temperatures 0.25 to 8 days. Eggs developed 5 h at 12°C before being tempered to holding temperatures.

Temperature (°C)		Days in holding temperature before transfer							
Holding	Test	0.25	0.5	1	2	4	5	7	8
	3			28		27	24		58
6[a]	6			80		83	87		92
	9			75		64	77		84
	12			64		70	66		91
	15			63		67	62		57
	3		40	19	9	16	16	64	
	6		68	89	80	75	98	96	
9[b]	9		82	84	87	90	96	98	
	12		79	82	72	80	97	98	
	15		76	80	79	98	95	98	
	3	26	29	31	24		82		
	6	75	70	93	82		98		
	9	82	89	91	84		98		
12[b]	12	76	86	93	94		100		
	15	76	81	82	81		98		
	3	29	45	20	18				
	6	82	70	57	65				
	9	71	67	76	75				
	12	64	65	76	76				
15	15[a]	70	81	83	84				
18		60	65	72	70				
3		6	23						
6		4	76						
9		70	77						
12		68	70						
15		67	70						
18[c]	18	71	73						

[a]Test temperature had a significant effect on normal hatch ($P < 0.01$, Friedman's test).
[b]Test temperature and embryo age had a significant effect on normal hatch ($P < 0.01$, Friedman's test).
[c]Embryo age had a significant effect on normal hatch ($P < 0.05$, Wilcoxon's signed ranks test).

survived transfer to and from 3°C better than yolk-sac larvae. Yolk-sac larvae did not survive when transferred to 3°C and survived only at 6°C when transferred from 3°C.

Larval Abnormalities

Various abnormalities were observed at hatching. At 3°C, from 8 to 10% of the larvae were malformed; another 2 to 5% remained partly in the egg shell and eventually died. At 6, 18, and 21°C about 2 to 3% of the larvae were abnormal; at other test temperatures less than 2% were abnormal at hatching. A higher incidence of malformations occurred among lots transferred from 6 and 18°C than among lots transferred from other temperatures. The more common abnormalities included lordosis, edema, and blood flow restrictions. All eventually resulted in death.

Table 5. Percentage survival of unfed northern pike yolk-sac larvae transferred directly to test temperatures 1 h after hatching. Larvae were held at test temperature for 6 days.

Hatching temperature[a]	Test temperature (°C)[b]						
(°C)	3[c]	6	9	12	15	18	21
3	100	80	0	0	0	0	
6	100	100	88	80	92	0	0
9	80	100	100	84	100	72	72
12	88	92	100	92	80	76	
15	100	100	80	96	100	92	44
18	80	84	100	100	92	88	80
21	94	68	92	92	84	80	80

[a]Hatching temperature had a significant effect on survival of yolk-sac larvae ($P < 0.01$, Friedman's test).
[b]Test temperature had a significant effect on survival of yolk-sac larvae ($P < 0.01$, Friedman's test).
[c]At 3°C all yolk-sac larvae died before becoming free swimming.

Discussion

The observed hatching temperature range (3.1 to 20.9°C) and lethal temperatures (3 and 24°C) are probably an accurate representation of the true temperature tolerance range for embryos of northern pike. The range was the same in all experiments even though fertilization temperature, egg source, age before transfer, and treatment were different. The observed hatching range was consistent with the ranges reported by Swift (1965) from England, Lillelund (1966) from Germany, and Hokanson et al. (1973) from Minnesota. Although some hatching occurred at the lower lethal temperature of 3°C, larvae did not survive if left at 3°C. Hokanson et al. (1973) observed that yolk-sac larvae hatched at 3°C were all abnormal and Lillelund (1966) indicated that all yolk-sac larvae died soon after hatching at 3.7°C. For normal hatching and survival of larvae, water temperature should be 6°C or more. Under field conditions, 5°C approached the lower tolerance limit of artificially fertilized pike eggs (Hassler 1970).

The embryo age (developmental stage) before temperature change influenced the percentage hatch but did not affect the hatching range. When normalized hatch was compared, embryos that developed for 2 h before temperature change had significantly lower hatches at all test temperatures, and their optimum hatching range and TL50's were narrower than for embryos that developed for 5 h or more before temperature change. Embryos were most sensitive (indicated by low hatching success) to temperature change and handling within the first 5 h of development. Earlier work with artificially fertilized pike eggs incubated on the spawning ground

(Hassler 1970) indicated that (1) mortalities were higher when water temperature reductions occurred during the first 3 to 5 days of incubation and not later, and (2) that pike eggs fertilized at higher, less variable temperatures later in the spawning season had a higher percentage hatch. Hokanson et al. (1973) found that the number of embryos hatching was higher for eggs that had developed for 5.5 h before temperature change than for those developed for only 0.25 h. Anwand (1965) indicated that pike embryos are extremely sensitive to compression and fall impact during the first hours after fertilization.

In the transfer experiment, further development before temperature change after embryos were 5 h old increased the hatch only from certain temperatures. For example, the percentage hatch was not increased when embryos were transferred from 6, 15, and 18 °C, even up to 8 days after fertilization. In other transfers, the percentage hatch increased only after embryos developed for 5 days at 9 °C before transfer and for 2 days at 12 °C. In all transfers, the percentage hatch was higher than the hatch in experiment 2, where temperature change occurred 2 h after fertilization.

The type of temperature change (shock or tempered) did not significantly influence the percentage hatch or the range of hatching temperatures. The hatch of shocked embryos 5 h old was essentially the same as that for tempered embryos 5, 24, and 36 h old before temperature change. Hokanson et al. (1973) found that there was no significant difference in hatch of shocked or tempered embryos subjected to temperature change 15 min after fertilization.

Embryos and yolk-sac larvae were very tolerant of temperature change within the optimum hatching range (6-21 °C). Embryos survived transfer from and to 3 °C better than yolk-sac larvae. Yolk-sac larvae transferred from 3 °C survived only at 6 °C and none from any holding temperature survived to the free-swimming stage when they were transferred to 3 °C.

The results indicate several factors that could be useful in fish culture and research. For example, maximum hatching of pike embryos in a hatchery or laboratory can be expected at a constant incubation temperature of 9 to 12 °C. Field stripping of eggs should not be done until water temperature has reached at least 6 °C, and preferably 9 °C. Additionally, fertilized eggs should be held at fertilization water temperature for at least 5 h before transfer or temperature change. Hassler (1970) and Lillelund (1966) both demonstrated that older embryos were more tolerant of temperature change. Also, if eggs are incubated at temperatures above 15 °C, larval abnormalities would be expected to increase and premature hatching might occur, precluding functional attachment of yolk-sac larvae to the substrate. Royer (1971) noted that pike fry stocked in a management marsh suffered a 40% mortality after 18 h. He suggested that since the fry showed no tendency to adhere to vegetation with their adhesive papillae and sank to the bottom, they may have settled where hydrogen sulfide or low oxygen was toxic. If eggs are incubated at 6 °C or less, mortality also will be high. Walker (1968) found that higher mortalities and weak larvae resulted from abnormally low hatchery water temperatures.

Acknowledgment

I thank the personnel of the South Dakota Department of Game, Fish and Parks for providing running-ripe pike.

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Accepted 5 March 1982.

Millimolar Quantities of Sodium Salts Used as Prophylaxis During Fish Hauling

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ABSTRACT: Rainbow trout provided with chronic dorsal aortic cannulae were placed in a tank with uncannulated fish at a density of 48 kg/m³ (3 lb/ft³). Following 4 h of simulated hauling conditions the principle blood buffer, HCO_3^- (i.e., 90% of arterial plasma total CO^2 content) was reduced by 22% and after recovery for 2 h arterial pH and HCO_3^- were significantly below pre-haul levels. Introduction of either NaCl or, in particular, Na_2SO_4 or $NaHCO_3$ into the hauling water (final concentration, about 10 m*M*) helped ameliorate the acid-base disturbances associated with handling and hauling. The addition of $NaHCO_3$ or Na_2SO_4 totally offset the decrease in blood buffering capacity associated with fish handling and hauling; in fact, a significant increase in blood buffering capacity was evident 2 h after fish were returned to the raceway. Addition of Na_2SO_4 or $NaHCO_3$ to water during routine fish hauling may help to reduce the effects of handling, crowding, and external stressors.

The ability to transport large quantities of fish from one location to another (e.g., from hatchery to lakes or streams for stocking) with low mortality has always been one goal of fisheries research. With increasing costs it has become even more desirable to transport as many fish per load as possible. In Colorado, where rainbow trout (*Salmo gairdneri*) are transported routinely from rearing units in one part of the State to stocking sites hundreds of miles away, hauling times of 4-6 h are not uncommon; less frequently, hauls of 24 h may be required. Adequate aeration and relatively constant water temperatures have proven essential to achieving low mortality during transport; however, we wanted to determine if any additional practical measures could be taken to further decrease hauling mortalities, so that even higher hauling densities might be possible.

Crowding produces stress in fish (Wedemeyer 1976) as do chasing, netting, air exposure, and struggling (Hattingh and Van Pletzen 1974; Chavin and Young 1970; Wedemeyer 1976; Wydoski et al. 1976). Consequently any effort to increase hauling density should attempt to minimize stress or the consequences of stress.

Hattingh and Van Pletzen (1974) observed a decrease in blood pH in the mudfish (*Labeo umbratus*) after capture and transport, noting that in extreme cases pH was still depressed 2-3 days after hauling. Wood et al. (1977) suggested that in addition to a breakdown in oxygen transport, decreased pH may produce other deleterious effects, such as increased blood viscosity due to erythrocytic swelling and reduced myocardial contractility. According to Jonas et al. (1962), if lactic acid production is severe enough to depress the pH into the range of 6.8-6.9 (11-14°C) mortality will result. Although a severe depression in blood pH may result in mortality, it has also been demonstrated that sublethal depressions in blood pH due to elevated environmental CO_2 result in decreased disease resistance in the channel catfish (*Ictalurus punctatus*; Walters and Plumb 1980). Thus, even if hauling does not produce a lethal depression in blood pH, it may still be desirable to minimize acid-base disturbances to reduce long-term mortality. If the stress associated with hauling large quantities of fish cannot be avoided, it might at least be possible to minimize the acid-base disturbances associated with hauling and stocking.

In goldfish (*Carrasius auratus*) that have been transferred to water lacking a permeant anion (sulfate substituted for chloride), the blood pH is significantly elevated (de Renzis and Maetz 1973). This elevated pH is correlated with elevated total carbon dioxide levels in the blood, apparently the result of an inhibition of the exchange of Cl^- and HCO_3^- that normally occurs across the fish gill (de Renzis and Maetz 1973; Haswell et al. 1980). In trout, pharmacological inhibition of the branchial exchange of Cl^- and HCO_3^- exchange also results in elevated blood pH (Haswell et al. 1980; Perry et al. 1981). Recently Perry et al. (1981) demonstrated that addition of $NaHCO_3$ and Na_2SO_4 to water also results in blood alkalosis in rainbow trout. The objective of our investigation was to ascertain if millimolar concentrations of

various sodium salts would eliminate, or at least ameliorate, the metabolic acidosis associated with the handling and hauling of rainbow trout.

Materials and Methods

In the present study we used rainbow trout weighing 0.7-1.0 kg. Although these fish were much larger than the rainbow trout typically used for stocking, they were available, and their size greatly facilitated surgical procedures and enabled repeated blood sampling. It should be pointed out, however, that transportation of larger numbers of smaller "stock size" fish would be associated with higher metabolic rates (oxygen consumption and ammonia production), which could lead to a greater degree of hauling stress. Fish were maintained in concrete raceways at the Bellvue Research hatchery at a water temperature of 11-12°C and not fed for at least 2 weeks before the studies began.

Trout were anesthetized with MS-222 and a dorsal aortic cannula was inserted, as described by Smith (1978). When a blood sample was not being drawn, the catheter was plugged and filled with heparinized Cortland saline solution (Wolf 1963). The fish were held in individual cylindrical wire cages. Fish in their cages were returned to the raceway and secured to the sides of live boxes suspended in the raceway. The catheter was led out of the cage so that sampling was routinely possible without handling or visually disturbing the fish.

We used two identical aluminum fish-hauling tanks, each containing about 425 L (112 gal) of water. Water in the hauling tanks was vigorously aerated and the temperature was maintained at 10-11°C by adding ice as required. In addition to the caged, cannulated trout, enough trout were added to yield a final population density of about 48 kg/m³ (? lb/ft³) in each tank. This value was chosen as representative of the hauling densities used in the State of Colorado. Generally, this density resulted in between 18 and 22 trout per tank. To ensure complete mixing, the salts were added to the tanks 30 min before any fish were added. Dissolved oxygen and ammonia levels were monitored in hauling water during the experiments. Water quality at the Bellvue research hatchery is characterized (in milligrams per liter) as follows: Ca^{+2} = 90; Mg^{+2} = 16; Na^{+} = 30; K^{+} = 1.6, HCO_3^{-} = 200 and Cl^{-} = 0.15.

Although a rise or fall in blood pH away from "normal" values indicates an acid-base disturbance, such a disturbance may occur without any apparent change in pH. Acid-base disturbances can be characterized by changes in "alkali reserve," as determined by actual or relative increases or decreases of alkali in body fluids in proportion to the content of acid. Hence, there need not be an actual change in the pH of body fluids; e.g., in a compensated acidosis the pH of body fluids could be normal (compensation would be achieved by enhanced respiratory excretion of carbon dioxide, use of alkaline reserves of body fluids, or excretion of a more acidic urine). Commonly, more than one compensatory mechanism comes into play. Bicarbonate is the principal buffer (or alkali reserve) in blood. At physiological pH and ionic strength, more than 90% of total CO_2 in solution is as bicarbonate; thus our measured arterial plasma total carbon dioxide ($TaCO_2$) is a reasonable approximation of plasma HCO_3^- concentrations. Although HCO_3^- values can be calculated by using the Henderson-Hasselbalch equation, conversion of $TaCO_2$ values to HCO_3^- does not change any conclusions and HCO_3^- values are therefore not presented.

Blood was analyzed for arterial pH (pHa), arterial partial pressure of oxygen (PaO_2) and of carbon dioxide ($PaCO_2$), arterial plasma total carbon dioxide content ($TaCO_2$), osmolality (mOsm), plasma glucose, and hematocrit. Values for pHa and PaO_2 were obtained from whole blood, using a blood-gas analyzer (Instrumentation Laboratories Micro 213) calibrated at ambient water temperatures. About 200 µL of blood was centrifuged immediately and the plasma was analyzed for $TaCO_2$ (Corning model 960 CO_2 analyzer) and osmolality (Wescor 5100B water vapor pressure osmometer). Calculations for $PaCO_2$ were performed by using the Henderson-Hasselbalch equation given by Perry et al. (1981). After determination of hematocrit values, plasma was separated and glucose determined with glucostat reagents. Water PO_2 was monitored with the blood-gas analyzer and ammonia with an ammonia-sensing electrode.

After allowing 48 h for recovery from the surgical procedure, initial blood samples were obtained. Caged fish were transferred individually to one of the two hauling tanks containing previously transferred, uncannulated, uncaged trout. To minimize the chances that a fish would struggle and dislodge its catheter, transfers were performed rapidly and fish were not out of water for more than 5 to 10 s. When a caged fish was secured in a hauling tank, a time-zero blood sample was taken and after this sample had been obtained the next fish was transferred, and so on. Fish were held in the hauling tanks for 4 h and then returned to the raceway; blood was taken again after about 2 h and the analyses of these samples are referred to as recovery values.

Commercial baking soda was used for $NaHCO_3$; reagent grades of NaCl and Na_2SO_4 were used. When used, salts were added to obtain a final concentration of 10 m*M* in the water in the hauling tanks. Because only two tanks were available, only two trials were conducted. In trial 1, untreated control fish were held in hauling tank 1 and fish in NaCl-treated water were held in tank 2. During trial 2, fish were placed in hauling water treated with either $NaHCO_3$ or Na_2SO_4.

Data were analyzed by using a paired *t*-test, with 0.05 as the limit for statistical confidence. For purposes of the *t*-test, all pH values were converted to H^+ and subsequently reconverted to pH.

Results and Discussion

Mean values (±SD) for the measured blood variables for 18 cannulated and caged rainbow trout resting in the raceway were as follows: pHa 8.036 ± 0.073; $TaCO_2$ 14.6 ± 4.8 m*M*;

PaO_2 117 ± 17 mm Hg; osmolality 285 ± 14 mOsm/kg H_2O; glucose 91 ± 25 mg %; hematocrit 21 ± 8%. Placing trout in wire cages did not have any obvious effect on blood acid-base status, since the values we obtained were generally similar to other published values for rainbow trout at 10-12°C (Cameron and Randall 1972; Randall and Cameron 1973; Haswell and Randall 1978). Further, the use of cages did not appear to hinder oxygen transport (i.e., uptake): the values for PaO_2 ranged between 112 and 125 mm Hg and inspired oxygen tensions fluctuated between 130 and 135 mm Hg, depending on daily conditions. Values for blood osmolality and hematocrit were also comparable to those for uncaged trout. Although quite variable, blood glucose levels (90 mg %) were within the normal range for trout (Wedemeyer 1972).

Values for blood variables for six trout before and immediately after transfer to the hauling tank and after a simulated haul are given in Table 1. Transfer of fish from the raceway to the hauling tank invariably resulted in an initial reduction in PaO_2 — undoubtedly a consequence of air exposure. However, during the simulated haul, water PO_2 remained near saturation and blood oxygen tensions at 2 and 4 h into the haul were not significantly different from pre-haul levels (unpublished data). After 2 h of recovery, PaO_2 was not different from pre-haul values. The transfer (air exposure) also produced a significant decrease in pHa, but $TaCO_2$ was not significantly different from pre-haul values. Although pHa did not fall during the simulated hauling experiment, at recovery all fish showed a highly significant fall in pHa and $TaCO_2$.

Blood lactate levels were not determined; therefore the respiratory and metabolic contributions to acidosis cannot be determined clearly. However, the tanks were well aerated and CO_2 levels in the water did not rise; it seems likely, therefore, that the base deficit is due primarily to a metabolic acidosis. At recovery the depressed pH and the 23% drop in $TaCO_2$ below pre-haul values were indicative of increased blood acid buffering and would be consistent with a metabolic acidosis. Although pHa did not fall during the simulated haul, blood $TaCO_2$ was significantly reduced from controls at both 2 and 4 h (unpublished data). The observation that pHa did not fall while fish were in the hauling tanks despite a decrease in $TaCO_2$ is somewhat puzzling. Although a normal blood pH and reduced $TaCO_2$ are consistent with a respiratory compensated acidosis in air-breathing vertebrates, there is little evidence to suggest that fish use changes in ventilation to facilitate pH adjustments (e.g., see Randall and Cameron 1973). A possible explanation is that blood ammonia levels were elevated. During the simulated haul, ammonia levels rose and were in the range of 1 μg/mL at the end of the 4 h. This level of environmental ammonia has been demonstrated to be sufficient to elevate blood ammonia levels in rainbow trout (Fromm and Gillette 1968). Furthermore, the pH of hatchery water was in the range of 7.9 to 8.3; thus, with a pKa of about 9, dissolved ammonia levels would have been quite high. Blood pH would have risen if ammonia in the blood had been available to buffer hydrogen ions and form NH_4^+;

Table 1. Means for blood variables (± 1 SD given in parentheses) for six trout before and immediately after transfer to the hauling tank, and 2 h after a simulated hauling experiment.

Variable (and units)	Values		
	Pre-haul	Zero	Recovery
pH	8.025	7.752*	7.902**
	(0.07)	(0.19)	(0.1)
$TaCO_2$[a] (mM/L)	12.1	11.8	9.8*
	(3.7)	(2.3)	(2.9)
$PaCO_2$[b] (mm Hg)	2.56	5.57*	2.83
	(0.56)	(2.93)	(0.47)
PaO_2[c] (mm Hg)	120	40*	118
	(4)	(9)	(6)
Osmolality	276	277	288
(mOsm/kg)	(6)	(16)	(15)
Glucose (mg %)	95	105	71
	(26)	(35)	(13)
Hematocrit	21	22	22
	(4)	(7)	(8)

[a]Total carbon dioxide content of arterial plasma.
[b]Arterial partial pressure of carbon dioxide.
[c]Arterial partial pressure of oxygen.
*$P < 0.01$.
**$P < 0.02$.

this possibility is being investigated. In spite of the absence of pH effects while fish were in the hauling tanks, the simulated haul produced a significant acidosis that was evident at recovery (or at a time equivalent to the time of stocking in an actual hauling situation).

The addition of salts appeared to provide a measure of protection against depression of blood pH associated with the experimental haul. The addition of sodium sulfate and sodium bicarbonate appeared to be more effective than sodium chloride.

When sodium chloride was added to hauling water, the pHa was slightly but significantly depressed from pre-haul values after 2 h of recovery (8.042 ± 0.087 to 7.958 ± 0.089, $P < 0.02$). Although $TaCO_2$ levels fell during the simulated haul, this reduction was only 13% after 4 h in comparison with 22% for the untreated haul (Fig. 1). After 2 h of recovery $TaCO_2$ was slightly but significantly less than pre-haul values. Hence, a minor acid-base disturbance was evident during the simulated haul, although pHa was not greatly depressed after 2 h of recovery. As in the control haul, glucose, osmolality, and hematocrit did not change.

When fish were exposed to water in the hauling tank containing either Na_2SO_4 or $NaHCO_3$ the acidosis evident with untreated water at recovery was completely ameliorated by a rise in $TaCO_2$ levels; this trend is seen in Fig. 1, in which the $TaCO_2$ levels of the four groups are compared. At recovery, pHa was not significantly different from pre-haul values in groups of fish treated with Na_2SO_4 and $NaHCO_3$. In both groups glucose, osmolality, and hematocrit were not affected by the simulated haul.

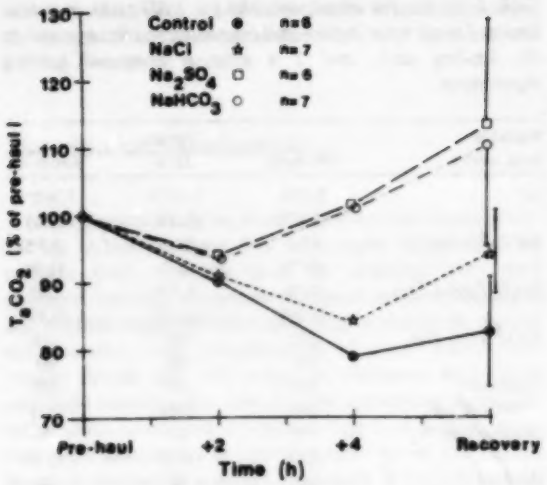

Fig. 1. Mean values of $TaCO_2$ (± S.D) for four simulated hauls and recovery periods. All values are expressed as a percentage change from pre-haul values. (For the sake of clarity, error bars are not depicted at +2 and +4 h.)

It seems safe to conclude that although all salts tested provided a measure of compensation for the decreases in HCO_3^- buffer reserve which occurred in association with this simulated hauling experiment, the sulfate and bicarbonate salts were superior in offsetting this loss. That is, both salts appear to maintain blood bicarbonate levels even in the face of increasing bicarbonate buffer demands. It is possible that elevated environmental sodium stimulates hydrogen efflux, and the non-permeant sulfate competes with environmental chloride inhibiting the HCO_3^- excretion that is normally coupled to chloride uptake. The external sodium concentration was about 10 m*M* in the NaCl-treated fish and about 20 m*M* in the sulfate-treated fish. Although a higher concentration of NaCl was not evaluated, the bulk of sodium uptake in the rainbow trout occurs by exchange diffusion and this sodium transporting mechanism is saturated at about 3 m*M* (Kerstetter et al. 1970). Consequently changes in sodium concentration between 10 and 20 m*M* probably have little effect on the sodium-coupled loss of hydrogen ions. Finally, when $NaHCO_3$ is used, the direct uptake of HCO_3^- from water to blood may occur. Whatever the mechanisms involved, the addition of low levels of sodium salts appears to reduce the acid-base disturbances associated with large-scale hauling of fish. All of the salts tested are readily obtainable and relatively inexpensive. Addition of $NaHCO_3$ to naturally acidic waters would produce elevated CO_2 levels, but in neutral or alkaline waters this salt is quite safe in millimolar concentrations.

Although our experiments are not representative of true hauling conditions, they are indicative of conditions that could occur. We did not attempt to duplicate road vibration or actual crowding, and we could not chase or net the catheterized fish. Thus, acid-base disturbances during an actual haul might be even more severe and the addition of salts of even greater benefit than our results indicate.

In conclusion it should be pointed out that Wedemeyer (1972) demonstrated that addition of NaCl and Ca helped offset ionic and osmotic disturbances occurring in the blood of fish during confinement. Although we did not evaluate blood electrolytes, we suggest that the use of Na_2SO_4 in hauling tanks may ameliorate ionic as well as acid-base disturbances.

Acknowledgments

We thank Thomas Powell and Lisa Rathburn for their technical assistance. This investigation was supported by Biomedical Research Support Grant and Faculty Research Grant funding from Colorado State University awarded to M. Stephen Haswell and by operating grants from the Colorado Division of Wildlife to Larry Harris.

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Accepted 8 April 1982.

Transportation Techniques for Blueback Herring

Clupeid fish frequently experience heavy mortality during hauling, which is to some extent counteracted when salt is added to the transport water (Collins and Hulsey 1964; Anderson 1968; Chittenden 1971; Stanley and Colby 1971; Bulak and Walker 1979) and, in some species, by adding anesthetics to transport water. However, although threadfin shad (*Dorosoma petenense*) and gizzard shad (*D. cepedianum*) were hauled successfully in 22 mg/L of MS-222 (Collins and Hulsey 1964; Anderson 1968), American shad (*Alosa sapidissima*) were intolerant of a 10-mg/L dose of MS-222 (Murai et al. 1979).

The present study was conducted to determine methods, concentrations, and kinds of anesthetics appropriate for use in hauling water for the successful air or truck transport of blueback herring (*A. aestivalis*) for as long as 10 h. The techniques we developed have been used in the transportation of blueback herring by air to Texas and by truck to Tennessee from South Carolina.

Blueback herring were collected by dipnet or castnet from Cooper River, Moncks Corner, South Carolina, at the catwalk of Pinopolis Dam and anesthetized immediately. Mean total lengths and weights of 20 males were 282 ± 2 mm and 209 ± 5 g, and of 20 females were 299 ± 2 mm and 278 ± 9 g.

The anesthetics we evaluated were quinaldine, tricaine methanesulfonate (MS-222), and etomidate (ethyl-methylbenzyl-imidazole-carboxylate), an experimental drug provided by Tavolek, Inc., Redmond, Washington. The anesthetized fish were used immediately in a series of tests to evaluate these anesthetics at various concentrations and a stocking density of 2 fish/10L of water. In pilot tests of higher stocking densities of 4 and 6 fish/10L of water, all fish died. Fish were also held without anesthetics (control) in transport water containing either salt (0.5‰) or Rila marine salt (10 ‰). The tests were made in double-lined plastic bags held in Styrofoam boxes. Air was forced out by deflating the bags to water level; oxygen was then added and the bags were sealed and covered. Boxes were then left unattended for 10 h. Bags were opened and fish behavior evaluated by the criteria described by McFarland (1960), with modifications (Table 1). Fish were acclimated to 11-13 °C and then released together in troughs for observation 10 h later to evaluate their behavior after anesthesia. We measured O_2, CO_2, total ammonia, and pH of the hauling waters by using test indicators or meters. In the later tests, the addition of ice to lower water temperatures, of 0.5 mg/L Furanace to combat bacteria, and of 20 g clinoptilolite to absorb ammonia were additives not quantitatively evaluated (the ice was added to the corners of the boxes containing the inflated bags).

Three additional tests were conducted to determine the best method for hauling herring by truck. Anesthetized fish were put into a rectangular tank containing 400 L of water with anesthetic and salt. A false top (Chittenden 1971) about 5 cm below the water line acted as a baffle and oxygen (3-5 L/min) was added through a porous pipe. Fish were hauled in the tank for 10 h, then removed to a holding trough for a 10-h observation period.

We used the most successful of the test methods to transport herring by air to a pond at Heart of the Hills Research Station, Ingram, Texas, and by truck to the Morristown (Tennessee) State Fish Hatchery.

Table 1. Classification of behavioral changes occurring in fish under anesthesia (modified from McFarland 1960).

Anesthesia Stage	Classification	Fish behavior
0	Normal	Highly excited; frantic reaction to external stimuli
1	Sedated	Decreased reaction to outside stimuli, but swimming ability retained
2	Loss of equilibrium	Able to stay afloat, but unable to maintain equilibrium
3	Light anesthesia	Complete loss of swimming ability, but able to move on the bottom (with tail movements)
4	Deep anesthesia	No movement on bottom, gill movement greatly reduced
5	Death	Gill movement ceased

Air Transport — Fish in the two salt concentrations (0.5% and 10 ‰) without anesthetics were dead after the 10-h post-anesthetic period.

Concentrations of quinaldine were tested at increments of 1 mg/L up to 10 mg/L. Fish did not survive at 1 and 2 mg/L, but did at the higher concentrations. Fish exposed to concentrations up to 7 mg/L exhibited loss of equilibrium; those exposed to higher concentrations were lightly anesthetized. After tempering, fish revived quickly and reverted to their normal behavior. Fish exposed to quinaldine were typically in poorer condition than those exposed to the other anesthetics. These fish had considerable hemorrhaging on their sides and at the base of their pectoral and pelvic fins. Water quality did not markedly improve as anesthetic concentrations increased; low pH (< 6.0), high total ammonia (> 1 mg/L), and CO_2 (> 20 mg/L) were typical.

Concentrations of MS-222 were tested at 5-mg/L increments up to 35 mg/L. Fish did not survive at the 1 mg/L concentration, but did at the higher concentrations. Fish exposed to concentrations up to 15 mg/L exhibited loss of equilibrium; those exposed to 20-25 mg/L were lightly anesthetized and those exposed to 30-35 mg/L exhibited deep anesthesia. All fish quickly revived during tempering. These fish typically exhibited less hemorrhaging than the fish exposed to quinaldine. Water quality was better at higher levels of MS-222. The deep anesthesia at 30-35 mg/L indicated that lethal concentrations were approached.

Etomidate was tested at increments of 0.05 mg/L from 0.1 to 0.40 mg/L. Fish survived at the lower concentrations, but mortalities occurred at 0.35 and 0.40 mg/L. Fish exposed to concentrations up to 0.25 mg/L exhibited light anesthesia; those exposed to higher concentrations went into deep anesthesia. After tempering, the fish were much slower to regain their normal swimming behavior than fish exposed to the other anesthetics. However, these fish had little hemorrhaging and a healthier appearance than those exposed to the other anesthetics. Water quality was better at the higher levels of etomidate.

Truck Transport — Quinaldine at a sedative dose (1 mg/L) used with 0.5% salt and a stocking density of 2 fish/10L was ineffective in tank transport. Survival after the 10-h test haul was 9%, and after the 10-h post-haul period it was 7%. Total ammonia was 0.5 mg/L and the pH was < 6.0 in the hauling water after the 10-h test haul. Low pH probably caused quinaldine to become ineffective.

Survival of fish hauled at a stocking density of 1 fish/10 L in an anesthetic dose of etomidate (0.1 mg/L) with 0.5% salt was 100% after a 10-h test haul, but only 48% after the 10-h post-haul period. Fish exhibited considerable loss of scales from lying on the tank bottom during the test haul. However, administration of a sedative dose of etomidate (0.05 mg/L) with 10 ‰ marine salt, 20 g clinoptilolite, and a stocking density of 1 fish/10 L resulted in high survival: after the 10-h test haul, survival was 100% and after the 10-h post-haul period it was 93%. Fish were able to maintain their proper posture during hauling and exhibited little scale loss or hemorrhaging after the test.

Field Evaluation — MS-222 (25 mg/L) or etomidate (0.2 mg/L) were chosen for air transport to Texas. Two fish were put into each bag containing water (12 °C; 10 ‰ salinity), anesthetic, 0.5 mg/L Furanace, and 20 g clinoptilolite. Bags were inflated with O_2 and sealed, and ice was added to the Styrofoam boxes. Seventeen boxes of fish with etomidate and three with MS-222 were flown to Kerrville, Texas. Fish were acclimated for 1 h by floating the bags in a pond at Heart of the Hills Research Station after the trip. Water temperature in the bags was about 11 °C, whereas in the pond it was 15 °C. All fish survived and appeared in good condition when released in the pond. After 1 month only two mortalities were noted. Spawning occurred and schools of small herring (about 5 cm total length) were seen in September 1980.

In truck hauling, anesthetized fish (1 fish/10 L) were loaded into the tank containing water (12 °C; 10 ‰ salinity), 0.05 mg/L etomidate, 0.5 mg/L Furanace, 500 g clinoptilolite, 10 mL Corning no-foam, and about 20 kg bagged ice. Fish were hauled from Moncks Corner to the Morristown State Fish Hatchery in about 8 h. Tank water had cooled to 9.5 °C. Ten of the 40 fish were above the false top and had considerable scale loss. Those below the baffle appeared in good condition; they were acclimated to 12 °C, put in a holding trough, and held for 1 month. One fish died on the third day, but all others survived the observation period.

Blueback herring can apparently survive the MS-222 levels used to transport *Dorosoma* spp. (Collins and Hulsey 1964; Anderson 1968), and are not as sensitive to this anesthetic as are American shad (Murai et al. 1979). If further tests show quinaldine to be an effective anesthetic for transporting herring, the concentrations required would be much higher than the levels (0.75-2.00 mg/L) typically used in fish

transport (McCraren and Millard 1978; S.K. Johnson, Texas A & M Extension Service, personal communication). Although etomidate appears to be a promising fish anesthetic, it is not yet commercially available and has not been registered for fishery use.

Further studies may show that stocking densities in both bags and tanks could be increased. Results of our present tests, however, confirm that every effort should be made to reduce stress and scale loss during the handling and transport of blueback herring.

Acknowledgments

We thank Jack D. Bayless, James S. Bulak, and Reginald M. Harrell of the Dennis Wildlife Center, South Carolina Wildlife and Marine Resources Department, Bonneau, South Carolina, and Max Sellers, Morristown (Tennessee) State Fish Hatchery, for their cooperation and assistance; Donald F. Amend, Tavolek, Inc., Redmond, Washington, who provided the experimental anesthetic (etomidate); and David J. Morris and Charles H. Fields, Jr., Texas Parks and Wildlife Department, who transported the fish by air.

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Accepted 1 September 1981

Portable Barge for Applying Lime at Community Lakes

Liming ponds containing acid bottom muds and soft water (< 20 mg/L alkalinity) to neutralize acidity, increase hardness and alkalinity, and increase fish production is a recommended practice in fish culture (Boyd 1979; Hickling 1962; and Neess 1946). After liming, phytoplankton growth, benthos production, and fish production are improved without increasing fertilizer application rates (Bowling 1962; Boyd 1974; Snow and Jones 1959; Thomaston and Zeller 1961). Liming increases the pH of bottom muds and the total hardness and alkalinity of the pond water, thereby increasing the availability of the phosphorus added in fertilizers (Boyd 1979).

Lime may be applied in several ways. The simplest liming method is spreader-truck application on dried pond bottoms, but this approach is not always possible. Small ponds may be easily limed with bagged or bulk limestone shoveled from a boat. Liming larger bodies of water, such as community lakes, requires large quantities of lime, heavy-duty equipment, and a large labor force working over an extended period.

We modified a fishing barge (5.5 × 2.4 × 0.35 m) to streamline the liming operation and thereby reduce the cost of distributing lime at seven community lakes. The work deck was constructed from treated lumber (5 × 20 cm). Flotation, supplied by polystyrene blocks, was attached below the work deck; total volume of the flotation was 1.85 m^3. A splash board (5 × 25 cm) the width of the barge was placed vertically 1.3 m forward of the stern to protect equipment from spray and lime slurry.

Lime was washed from the barge with a 7.6-cm discharge self-priming reciprocal pump powered by a 5-hp air-cooled gasoline engine. The pump was rated at 822 L/min at

3,600 rpm. We mounted the pump on the port side behind the splash board. The screened pump intake was mounted at the bow to reduce intake of lime slurry as the barge moved forward. The 7.6-cm discharge was reduced through a tee to two 3.8-cm ID threaded male ends to which two 3.1-m heavy-duty neoprene hoses were attached. The diameter of the stream of water from each hose was further reduced by a 1.6-cm ID outlet nozzle, 30.5 cm long. These reductions produced a water volume with sufficient pressure to wash large quantities of lime from the barge within a few minutes.

Outboard motors from 6 to 25 hp were used to power the loaded barge. The larger outboard motor was more efficient to operate because individual loads of lime could be applied in a shorter period.

A 5.2-m tandem boat trailer was modified to accept the polystyrene pontoons so that the barge could be trailered from lake to lake.

Bulk calcitic limestone was delivered to each lake and stored near the boat launching ramp to facilitate barge loading. Material and manpower costs were reduced by using bulk lime instead of bagged lime.

Lime was loaded on the barge with a tractor-mounted 0.08-m³ bucket. Loads were centered on the barge so a maximum volume of lime could be handled. An average of 0.7 t of lime was transported each trip. A slurry of lime and water was produced by flooding the center of each load. The slurry was then washed from both sides of the barge. Lime was applied to the entire lake surface; however, higher densities were applied in areas less than 2 m deep.

Optimally, the barge crew consisted of three members, a coxswain and two hose operators (Fig. 1). However, when manpower was limited, two crew members could operate the barge and handle the spray equipment. One equipment operator was needed to load the lime on the barge with a front-end loader.

About 560 t of agricultural limestone were applied to seven community fishing lakes ranging from 21 to 74 ha. The lime applications at all seven lakes required 260 man-hours. The total surface area limed with the barge was 785 ha. About 75% of the lime at each lake was applied by using the barge. As much as 134 t of lime were applied by barge to a single lake. Application rates ranged from 2.0 to 3.5 t/h.

Savings in time and money from using the barge and bulk lime were estimated to be about 4.1 man-hours and $57.00 per hectare, respectively.

Fig. 1. Lime being applied by barge at a community lake.

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Accepted 2 April 1982

Factors Affecting the Efficiency of Clinoptilolite for Removing Ammonia from Water

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ABSTRACT: Clinoptilolite, a natural zeolite, is efficient for removing ammonia from water. This study was done to evaluate its efficiency for removing ammonia under various conditions of water hardness, pH, and temperature, and different granule sizes. Ammonia removal efficiency was unaffected by variations in temperature, but decreased in hard waters, in waters of low and high pH, and with size of granule. Efficiency was optimum in water of 44 mg/L or less total hardness, near neutral pH, and for granules of 20 × 30 mesh size. Adsorptive capacity ranged from 3.42 to 9.12 mg of ammonia per gram of clinoptilolite.

Ammonia generally enters water systems as a pollutant, and may become toxic to fish if nitrification does not convert the reduced form to nitrite and ultimately to nitrate (Thurston et al. 1978). In fish culture systems, ammonia is excreted in the water as a metabolic by-product. The problem is potentially serious in hatcheries where water is reused or where ammonia accumulates as water flows through a series of raceways. Sublethal concentrations of ammonia may inhibit growth or increase the susceptibility of fish to other stress conditions (Spotte 1970). It is therefore imperative that lethal and sublethal concentrations of ammonia be avoided if survival and growth are to be optimum.

Methods for nitrogen removal from wastewater, reviewed by Reeves (1972), included three principal methods that could be adapted to fish culture systems: ion exchange, nitrification (biological filters), and air strippng. Of the three methods, ion exchange appeared to have the greatest potential.

Activated carbon effectively removed organic chemicals from water but was ineffective for removing ammonia (Dawson et al. 1976). On the other hand, Atkins et al. (1973) reported that carbon effectively removed ammonia from chlorinated water because chlorination apparently converts nitrogen-bearing compounds to organic forms such as chloramines. Since chlorine and the resulting chemical complexes are also toxic to fish, the procedure has little potential for use in fish culture.

Clinoptilolite, a natural zeolite and ion exchange resin, was found to be efficient for removing ammonia from secondary effluents in laboratory studies. Furthermore, in pilot plants, 97-99% of the ammonia was removed when effluents were passed through columns containing clinoptilolite (Mercer et al. 1970). Semmens et al. (1977*a*, *b*) demonstrated the efficiency of clinoptilolite for removing ammonia from water and described procedures for regenerating the resin. They found that regeneration is more economical and efficient if nitrifying bacteria are used to assist in the regeneration process. Later, Semmens and Porter (1979) demonstrated that biologically regenerated material was stable and effective, and that it removed 90-97% of the ammonia during 43 cycles.

Johnson and Sieburth (1974) reported that clinoptilolite was an effective adsorbent for ammonia in fresh water, but that its efficiency was markedly decreased in salt water. Because adsorpton efficiency is apparently influenced by chemical characteristics of water, we attempted to determine the efficiency of clinoptilolite for removing ammonia under various conditions of water hardness, pH, and temperature, and different granule sizes.

Materials and Methods

Clinoptilolite (4-mesh granules) was obtained from the Baroid Division of National Lead, Hecter, California. These granules were ground and sieved, using stainless steel screens with meshes of 8, 18, 20, 30, and 50 per square inch to yield mesh sizes of 8 × 18 (particle diameters of 990-2, 286 μm), 20 × 30 (508-813 μm), and 30 × 50 (279-508 μm). For example, the 8- × 18-mesh granules would pass through 8 openings per square inch but not through 18 openings per square inch. Sieved granules were washed with deionized water, dried in an oven, and stored until needed. Columns were prepared by placing 60 g (about 15 cm) of the resin in glass tubes (2.54 cm ID) containing glass wool plugs to retain the adsorbent. Columns containing new (nonregenerated) resin were used for each test.

Ammonia solutions were prepared by dissolving ammonium chloride (NH_4Cl) in water; concentrations of ammonia were calculated on the basis of available nitrogen. Measured quantities of test solutions were passed through the columns at a flow rate of 25 mL/min. Concentrations of ammonia in the column effluents were determined with an Orion Model 407 specific ion meter. The end point for effective adsorption

was arbitrarily selected as 50% of the concentration of the stock solution (i.e., beyond that point less than half of the ammonia would be removed).

The effects of water hardness, pH, and temperature on removal efficiency were determined by preparing solutions of ammonium chloride in waters of different hardness and pH according to standards recommended by ASTM Committee E-35 on Pesticides (1980). Selected temperatures were obtained by immersing ammonia solutions in water baths. Water hardness tests included water-ammonia solutions of 12, 44, 170, and 300 mg/L as $CaCO_3$, and the pH of those solutions was maintained at a nearly constant 8.2 by adding 384 mg/L of sodium bicarbonate to each test water (Marking 1975). Selected pH's of 6.5, 7.5, and 9.5 were prepared and maintained with chemical buffers.

Adsorption Efficiency

Temperature

Ammonia removal efficiency was not affected by temperatures of 12 or 22 °C (Table 1). McLaren and Farquhar (1973), who studied certain factors suspected to affect ammonia removal efficiency of clinoptilolite, reported a small change in adsorptive capacity as temperatures increased from 2 to 23 °C, and recommended that the data for 23 °C provided a good estimate for adsorption over a wide range of temperatures. Also, their study suggested that the initial ammonium ion concentration had the greatest effect on removal, and that high flow rates decreased adsorptive capacity.

Water Hardness

The efficiency of clinoptilolite decreased as water hardness increased (Table 1). Only about one-half of the ammonia was removed in very hard water (4.30 mg of ammonia per gram of clinoptilolite) as compared with very soft or soft water (9.12 and 8.55 mg/g). Other investigators have noted that the presence of calcium and magnesium ions substantially reduce ammonia adsorption by clinoptilolite. Koon and Kaufman (1971) showed that an early breakthrough of ammonia occurs when the ions are more abundant. Semmens and Porter (1979) also reported poor column performance correlated with high calcium and magnesium concentrations in the regenerant. Smith et al. (1981) reported that unregenerated clinoptilolite granules decreased the calcium concentration and total hardness of water even though clinoptilolite is more selective toward ammonia than toward calcium and magnesium.

Hydrogen Ion Concentration (pH)

The efficiency of clinoptilolite decreased in water of low pH (Table 1); at pH 6.5 the column removed 3.42 mg of ammonia per gram of resin, as compared with 7.85 mg at pH 7.5. Efficiency appeared to peak in neutral or near neutral water and diminish toward either end of the pH scale. Adsorptive capacity of clinoptilolite at various pH values has not been reported in the literature, and we investigated only the range that might be found in water in which fish are cultured. In the regeneration process for clinoptilolite, ammonia is efficiently removed from the resin with a brine solution at pH 11 to 12, and we suspect that acid solutions would also remove ammonia from the resin.

Table 1. Ammonia removal capacity of a 15-cm column (60 g dry weight) of clinoptilolite under various conditions, using a constant flow rate of 25 mL/min.

Test variation and temp (°C)	Hardness (mg/L)	pH	Mesh size	Adsorptive capacity (mg NH_4-N/g of clinoptilolite)
Temperature				
12	44	7.5	20 × 30	7.85
22	44	7.5	20 × 30	8.32
Hardness				
12	10	8.0	20 × 30	9.12
12	44	8.0	20 × 30	8.55
12	170	8.0	20 × 30	5.32
12	300	8.0	20 × 30	4.30
pH				
12	44	6.5	20 × 30	3.42
12	44	7.5	20 × 30	7.85
12	44	9.5	20 × 30	6.17
Granule size				
12	44	7.5	8 × 18	5.37
12	44	7.5	20 × 30	7.85
12	44	7.5	30 × 50	8.02

Granule Size

Large granules were less efficient than smaller ones. The removal rate was 5.37 mg/g for 8- × 18-mesh granules (Table 1), as compared with about 8 mg/g for smaller granules. Although 30- × 50-mesh granules were slightly more efficient than 20- × 30-mesh granules, the smaller granules tended to restrict flow and to clog more readily than large granules; they may therefore be impractical for filtering water used in fish culture. The 20- × 30-mesh granules were the most efficient of the three sizes tested. Smith et. al. (1981), who used clinoptilolite columns to remove ammonia from fish culture water, reported that extensive backwashing was required to prevent fouling of the 20- × 42-mesh granules they used.

Efficiency Considerations

Because the efficiency of clinoptilolite is influenced by many factors, planners and designers of fish culture and water reuse systems should be aware that ammonia removal efficiency of clinoptilolite decreases appreciably for water that is increasingly acid or alkaline and for water of increasing hardness. Granule size is important because efficiency decreases if the granules are too large, and water flow rate is generally impeded if the granules are too small. Even so, the

results of recent studies are encouraging. Bruin et al. (1980), who quantitated the volume of ammonia produced by fish and correlated that amount with clinoptilolite removal efficiency, were able to demonstrate acceptable growth of salmon in water effectively treated with the resin. Lorenzo et al. (1981) demonstrated the potential usefulness of clinoptilolite in water treatment systems by showing that the exchange resin remained effective for removing ammonium and phosphate ions from municipal effluent after about 500 regenerations. In their study, ammonia complexes of $MgNH_4PO_4$ and NH_4NO_3 were extracted from the clinoptilolite and used for agricultural fertilizer, and the brine solutions used for regeneration were suitable for reuse.

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Accepted 29 March 1982

Fin Erosion and Culture-related Injuries of Chinook Salmon Raised in Floating Net Pens

Fin erosion and other minor injuries are often prevalent in salmonid fishes cultured in hatcheries. Fin erosion frequently involves almost all cultured fish held in confined rearing facilities and is commonly used as a field indicator of the domesticated origin of a fish (Novotny 1980). The rearing of salmonids in floating net pens has been practiced since 1967 in the Pacific Northwest (Novotny 1975), and net-pen culture of Pacific salmon (*Oncorhynchus* spp.) has since evolved into a multimillion dollar industry, in which product quality is a prime market consideration (Wagner 1972; Lindbergh 1973; Woodin 1981).

Because floating net pens provide a different type of rearing environment for salmonid culture, and injuries may detrimentally affect the market value of salmon, the present

Table 1. Initial and final average weights and release times of chinook salmon reared in floating pens in Puget Sound, 1971-73.

Year and location	Number of pens	Average weight (g)		Rearing time (days)	Final densities in pens (kg/m³)
		Start	Finish		
1971-72					
Big Beef Creek	4	12.3	121.2	228	14.9-18.7
Clam Bay	4	12.8	145.4	214	15.7-16.6
Squaxin Island	4	12.5	168.0	246	14.0-20.8
1972-73					
Friday Harbor	3	5.8	83.9	249	10.0-13.3
Clam Bay	4	4.8	92.9	256	12.5-13.9
Squaxin Island	4	5.6	127.5	260	23.8-28.2

study was conducted to (1) examine the type and extent of injuries in chinook salmon (*O. tshawytscha*) in saltwater pens, (2) relate rearing injuries to density of fish, (3) relate rearing injuries to time of year, and (4) relate injuries to periods when fish are under disease stress.

Chinook salmon reared in floating net pens were of the Finch Creek stock, hatched and reared at Hoodsport Hatchery, a Washington Department of Fisheries hatchery near Hoodsport, Washington. Salmon from successive year classes were ultimately transferred in 1971 and in 1972 from the hatchery to floating pens at four sites in Puget Sound: Squaxin Island (southern Sound), Clam Bay (central Sound), Big Beef Creek (a brackish-water pond near Hood Canal), and Friday Harbor (San Juan Islands).

The cuboidal 2.4-m floating net pens were made of 6.4-mm square-mesh nylon netting. Pen shapes were held relatively stable by square 2.4-m frames of weighted polyvinylchloride pipe. Because nets were secured to floating frames above the water line, actual total rearing volume was 12.7 m³ per pen. Overhead netting restricted bird predation on fish in pens.

The average weight of the salmon was 11.9 to 12.8 g when rearing began in pens in July 1971, and 4.8 to 6.2 g when rearing began in May-June 1972. Fish were raised in the saltwater pens continuously until they were released (Table 1).

Of 22,647 salmon released in 1972 and 23,555 released in 1973, 12,251 (54%) and 2,634 (11%), respectively, were examined for fin erosion, culture injuries, and abnormalities. An additional 2,000 salmon from two pens at Clam Bay were examined and released in 1972; however, because of extensive injuries from two severe attacks by birds, the injuries and deformities were not considered representative and these data were not included in the present analysis.

Splitting and erosion of fins was the most common abnormal feature of pen-cultured chinook salmon; erosion was detectable in most fish and was moderate to extensive in 11.3% in 1971-72 and 15.8% in 1972-73 (Table 2). In particular, caudal and dorsal fins had extensive deterioration and hemorrhaging in 10 to 24% of the fish at individual sites. Severe splitting or erosion of the caudal fin was noted in 3 to 8% of the fish and hemorrhaging on fins was apparent in up to 13% of the fish at individual locations. Some "snubbing," or erosion of the dorsal fin, was noted in more than 90% of the salmon. Eye injuries were present in 0 to 1.4% of fish at individual sites. Other conditions noted included snout and head wounds, net marks, broken opercula, excessive scale loss, body lesions, fungus, and scars from attacks by birds.

Final rearing densities in 23 net pens ranged from 10.0 to 28.2 kg/m³. Rearing injuries at the completion of all experiments ranged from 14 to 39% of the salmon in a single pen. Regression analyses showed no significant correlation between final rearing densities and percentage of injuries. When computed just for densities less than 18 kg/m³, correlation was slightly better, but there was no significant correlation when final densities were used as indicators.

As was expected, fin erosion and rearing injuries in cultured salmon increased with time, although there was considerable variability. For most pen groups, the frequency of injuries and abnormalities exceeded 20% in late summer to mid-fall. Examinations of pooled growth and injury data from two randomly selected pens from each season at the

Table 2. Type and extent (%) of fin erosion, culture injuries, and abnormalities in pen-cultured chinook salmon (N = 14,885).

Injury or abnormality	Year and (in parentheses) number of pens examined	
	1971-72 (16)	1972-73 (12)
Fin damage[a]	11.3	15.8
Hemorrhaged fins	7.3	8.3
Eye injuries	1.0	0.5
Fungus	0.2	0.1
Abnormal coloration	0.2	0.1
Net marks	0.2	0.1
Snout-head injuries	0.1	0.4
Vertebral abnormalities	<0.1	0.1
Broken operculum	<0.1	0.2
Excessive scale loss	<0.1	0.1
Other	0.3	1.1

[a]Moderate to extensive splitting or deterioration (not including hemorrhaging).

Squaxin and Clam Bay sites showed that frequency of injuries in net pens increased after July. This increase did not appear to be related to density per se, although biomass in some pens doubled during this period. Impetus for this increase may have been, in part, stress caused by outbreaks of vibriosis. Despite the variation among pens, even at the same site, rearing injuries and abnormalities never exceeded 5% before August, and never dropped below 10% in any pen after mid-September.

Salmon were under stress at the time of initial transfer to pens and for brief periods when they were sampled for growth measurements. The principal periods of longer term stress were during outbreaks of vibriosis. Records of frequency of injuries before and after such outbreaks were available for seven periods at four locations. Although there was an apparent cause-and-effect relation between injuries and four of the outbreaks, when all records were combined there was no significant difference ($t = -1.11$) between frequency of injuries and outbreaks of vibriosis.

Erosion of dorsal caudal fins in salmonids is widespread in high-density culture operations. Davis (1967) attributed higher frequencies of such fin erosion to overcrowding. Outbreaks of the intensive culture disease, fin rot, have also been correlated with population density, although there is apparently no correlation after the disease has been initiated (Schneider and Nicholson 1980). Final rearing densities in the present study, however, were not unusually high in comparison with those employed in most later commercial net-pen operations described by Novotny (1975).

Though I found little correlation between frequency of rearing injuries and final rearing densities (somewhat different results from the metal cage experiments of Boydstun and Hopelain [1977], and possibly reflecting the limited impact of densities of 20 kg/m³ in the Puget Sound experiments), there appeared to be a relation between duration of rearing (interval rearing densities) and frequency of rearing injuries. This relation may, however, be a reflection of optimal temperatures for growth (Brett 1952), which result in increased size of fish. Such crowding can lead to optimal conditions for outbreaks of vibriosis, which increase stress. The severity of vibriosis outbreaks depends partly on early detection and effective therapeutic treatment. The effectiveness of oral treatment of vibriosis with antibiotics (the standard procedure during the period of testing) is unpredictable. Different strains of *Vibrio anguillarum* are involved in these outbreaks, even in the same locale, and some strains are resistant to oxytetracycline and sulfa (Novotny 1978). Hence the effectiveness of detection and treatment techniques varied among outbreaks, as did the extent of stress and injuries.

Although erosion or snubbing was quite common, severe snubbing occurred only in 10-24% of the fish at individual sites. Scale loss during net-pen rearing was excessive in less than 0.1% of the fish, and hemorrhaging of fins never exceeded 13% of the fish at any one location. As a consequence, I conclude that rearing injuries in pen-cultured chinook salmon can be kept at acceptable levels for densities up to 20 kg/m³ if care is taken to minimize stress during periods of high exposure to vibriosis.

Acknowledgments

Principal financial support for these investigations was provided by Washington Sea Grant project R/A-2, part of the National Sea Grant Program. Additional funding came from Seattle City Light, Snohomish County Public Utility District, and the Small Tribes Organization of Western Washington. I gratefully acknowledge the assistance of biologists with the National Marine Fisheries Service at the Manchester aquaculture facility and Ernest O. Salo, Fisheries Research Institute, University of Washington. I thank Jon G. Stanley, Maine Cooperative Fishery Research Unit, and Roger P. Dexter, Craig Brook National Fish Hatchery, for reviewing the manuscript.

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Accepted 2 April 1982

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Evaluation of Fluorescent Pigment for Marking a Scaleless Fish, the Brook Stickleback

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ABSTRACT: More than 93% of brook stickleback (*Culaea inconstans*) retained some fluorescent pigment for at least 8 days; some marked fish were recovered as long as 11 months after release. Immediate mortality due to marking adults was usually less than 3%; juvenile mortality, however, ranged from 23 to 68%. After periods of several months there is probably some mark loss and some additional mortality due to marking.

Studies of population dynamics, dispersal, and other aspects of the life histories of fishes frequently require the release of marked individuals. Small species usually must be given a common (batch) mark and often large numbers of individuals must be marked at a time. Fluorescent pigment marking (Jackson 1959) has proven to be an excellent technique for mass marking large numbers of fish (Strange 1977; Phinney and Mathews 1969). This method has been used on a variety of scaled species and on some salmonid fry before they develop scales (White 1976). The only previous mention of the use of this method on species which never develop scales is by Jackson (1959), who gave no results of his experiments on hornpout (*Ictalurus* sp.).

In the present report we describe the results of applying the fluorescent marking technique to a small, scaleless species, the brook stickleback (*Culaea inconstans*). Marking success, ease of detection of marks on dead and live fish, and mortality due to marking, are reported and the effect of marking on the growth of unconfined fish is described.

Materials and Methods

Fish were obtained from lake 200 in the Erickson-Elphinstone experimental aquaculture area of Manitoba. Fish were caught in unbaited "Gee" minnow traps and either marked immediately or held (for as long as 3 days) in cages consisting of 2-mm-mesh nylon "Ace" netting on floating 1.25-m^2 frames. After marking, fish were returned to the cages and then released the following day. A group of marked and unmarked controls were caged for an additional 6 to 8 days after marking to assess mortality due to marking, the success of marking, and mark retention.

For marking, 200-400 fish were placed in a wooden cradle 38 × 55 cm with a flat bottom of 1.5-mm-mesh plastic screen. The sides (15 cm high) allowed the cradle also to serve as a small floating cage which, with its contained fish, could be placed in a tub of water so that the fish were removed from water for only the few seconds required to apply the pigment. Pigment was obtained from Scientific Marking Materials, Seattle, Washington, and is advertised as being "mostly" between 50 and 350 μm in diameter. The pigment was applied with a sandblasting gun (Model 362-A, W.R. Brown Corp., Chicago, Illinois), operated by an air compressor. Pressures ranged from 690 to 552 kPa (application began at 690 kPa and halted when the pressure had fallen to 552 kPa). The fish-to-gun orifice distance was 25-28 cm. Batches of fish were normally sprayed with pigment and placed in water, sprayed a second time and again placed in water, and then sprayed a third time. The interval between applications was about 45 s — the time it took the compressor to restore the pressure to 690 kPa. This interval was advantageous because it allowed fish to clear some excess pigment from their gills and bodies between exposures and produced a redistribution of fish on the screen that reduced the chances of a given fish being shielded by others.

Fish were examined for marks by using long-wave illumination from a high-intensity ultraviolet lamp (model B-100A, Ultra-Violet Products Inc., San Gabriel, California) in a darkened room. Both preserved and live fish were checked for marks.

The effect of marking on growth was evaluated by comparing standard length, body weight, and condition factor ($100 \times \text{weight/length}^3$) of marked and unmarked fish taken from the lake in June 1980 and 1981, 1 month after marking and release.

Short-term Results

Marks were retained by 93% or more of the brook stickleback for at least 6 to 8 days (Table 1). Marked fish had pigment particles concentrated in the bony areas (i.e., the head, and especially the operculum, at the bases of the spines, and between the fin rays). The number of pigment particles per fish varied from 1 to more than 100.

Mortality due to marking ranged from less than 0.3% to a maximum of 68% during the first 24 h (Table 2). Mortality

Table 1. The percent of brook stickleback bearing marks 6 to 8 days after pigment application.

Year and month	No. of Fish		Fish marked (%)
	With mark	Without mark	
1979			
June	127	1	99.2
July	108	2	98.2
August	535	1	99.8
September	592	40	93.7
1980			
June	23	0	100.0
July	35	0	100.0
August	99	0	100.0
September	94	0	100.0
1981			
May	101	0	100.0
September	51	0	100.0

was clearly associated with fish age and environmental conditions: mortalities of adults marked in June and July 1979, May and June 1980, and May 1981 were lower than those of young of the year marked in August 1979 and in July and August 1980 during the first 24 h after marking. Death of young fish appeared to be the result of disease that probably was aggravated by excessive pigment damage to the soft integument, by high water temperature, and by crowding of the fish. In August 1980, fish were immersed in a malachite green solution after spraying (Phinney et al. 1967); however, survival was less than when this treatment was omitted. By September, when the fish were larger and probably had stronger integuments and water temperatures were lower, mortality declined. Like Hennick and Tyler (1970), we found that mortality was largely confined to the first 1 or 2 days after marking (Table 2).

The high mortality exhibited by juvenile brook stickleback may also occur to some degree in juveniles of other species (Andrews 1972). Because we did not experiment on juveniles with different pressures and distances we do not know whether mortality could be reduced by changes in the method of application.

Marking and handling had little effect on growth in either 1980 or 1981. After 1 month at large in the lake, mean standard length and body weight of marked fish were not significantly lower than those of unmarked fish taken at the same time. In 1980, however, marked fish had a significantly lower mean condition factor than unmarked fish (Table 3). Comparisons could not be extended beyond 1 month because of the timing of recruitment of unmarked juveniles and the post-reproductive mortality of the adults.

Long-term Results

Although relatively short-term mark retention and survival were satisfactory for adults in the present study, we found evidence suggesting a significant decline in long-term retention and survival. In a sample of 510 fish taken 1 week after marking in September 1980, we found 25 that were marked. In a second sample of 2,840 collected in May 1981, only 65 marked fish were present. The frequency of marked fish thus dropped from 5.1 to 2.2% [$\chi^2(1) = 11$, $P < 0.005$]. Correspondingly, we observed that 1 week after marking the above group, fish carried a mean of 72 (SD = 40.4, n = 74) pigment granules each, whereas 9 months later the mean had fallen to 33 (SD = 23.8, n = 73) — a significant decrease

Table 2. Percent mortality of brook stickleback attributed to marking. First night mortality is based on deaths among the total marked. Mortalities for subsequent nights are based on deaths (indicated in parentheses) among marked and unmarked controls, respectively.

Year and month	$\bar{X}$ fish weight (g)	Total marked (no.)	Nightly mortality (%)								No. of controls	
			1	2	3	4	5	6	7	8	Marked	Unmarked
1979												
June	1.7	1,503	1.5	1.4(2,0)	2.8(4,0)	1.5(2,0)	0.7(1,0)	0.3(2,1)	0(0,0)	0(0,0)	142	68
July	1.0	4,050	2.9	0(0,2)	2.3(4,2)	0.3(1,1)	0(1,3)	0.1(2,3)	—	—	118	183
Aug.	1.0	38,225	23.3	0.8(6,1)	0.5(3,0)	0(0,0)	0.2(1,0)	0(0,0)	0(0,0)	—	546	349
Sept.	1.1	29,687	8.8	0.6(4,0)	0.5(3,0)	0.3(2,0)	0(0,0)	0.3(2,0)	0(0,0)	0(0,0)	648	172
1980												
May	1.8	545	2.0	—	—	—	—	—	—	—	—	—
June	2.3	2,070	8.6	0(0,0)	0(0,0)	0(0,0)	0(0,0)	0(0,1)	—	—	23	23
July	0.9	12,602	58.1	13.9(15,1)	0(2,3)	0(0,0)	0(0,0)	0(0,0)	—	—	100	91
Aug.	1.0	12,347	68.0	18.2(19,1)	53.7(43,0)	32.3(12,0)	56(14,0)	9.1(1,0)	—	—	99	103
Sept.	1.2	4,063	3.8	0(0,0)	0(0,0)	4(4,0)	0(0,0)	0(0,0)	—	—	100	98
1981												
May	1.8	5,059	0.3	0(0,0)	0(0,0)	0(0,0)	0(0,0)	0(0,0)	—	—	100	25
Sept.	0.9	6,517	7.8	0(0,0)	0(0,0)	0(0,1)	0(0,0)	0(0,0)	—	—	51	51

Table 3. Effect of marking and handling on the growth of brook stickleback over a 1-month interval.

Year	Length (cm)				Weight (g)				Condition			
	$\bar{X}$	SD	No.	*P*	$\bar{X}$	SD	No.	*P*	$\bar{X}$	SD	No.	*P*
1980												
Marked	5.52	0.43	23		2.57	0.66	23		1.51	0.13	23	
				0.66			91	0.70	1.59	0.2	91	0.018
Unmarked	5.47	0.44	92		2.62	0.6						
1981							87		1.49	0.16	87	
Marked	5.15	0.31	87		2.04	0.39	0.03					0.99
				0.02	1.93	0.40	218		1.49	0.14	218	
Unmarked	5.05	0.36	218									

($t = 7$, $P < 0.001$). Migration was impossible and there was no recruitment at this time; thus, the implication is that fish lost pigment granules over time or that heavily marked fish did not survive as well as those less heavily marked, or both. (Over a 31-day period, however, the decline in the frequency of marked fish was not significant.) These problems may not be unique to species such as the brook stickleback. Previous studies have not compared the quality of marks over time, nor has long-term survival in the wild often been examined over a substantial portion of the life span. Serious mark loss in rainbow trout (*Salmo gairdneri*) was reported by Vashro (1978), however.

Comparisons with Scaled Species and Processing Problems

Although these scaleless fish were marked successfully, they retained substantially fewer particles per fish than a scaled species, the fathead minnow (*Pimephales promelas*), which we treated with the same technique and apparatus.

In contrast to the findings of Andrews (1972) and Phinney et al. (1967), we regularly observed pigment that had penetrated the cornea. Although the air pressures we used were lower than those of the workers cited, we probably achieved greater pigment impact force as a result of our short fish-to-nozzle distance. Some corneal damage may be inevitable if a scaleless species is to be adequately marked. Phillips and Barraclough (1977) also observed eye damage to 5% of the lingcod (*Ophiodon elongatus*) they marked.

When only a few small particles were present on the brook stickleback, processing of fish to identify marked individuals was relatively slow. The rate for dead brook stickleback in the present study was about 260/h, whereas Andrews (1972) reported handling fathead minnows at a rate of 2,000/h, a rate we find somewhat high on the basis of our experience with the same species. In our study some marked fish were not detected and some unmarked fish may have been incorrectly classified as marked due to natural fluorescence of bony parts, parasites, or contaminating stray pigment. Some of these problems of mark detection may not be restricted to scaleless species. Andrews (1972) noted some of the fathead minnows carried "very few" or only one granule and such fish would probably be subject to the same types of observer error that we experienced. Failure to detect all of the marked fish in a sample (a form of mark loss) may be a general problem with this technique that has not been recognized in previous studies.

Retention of only a few small particles caused their detectability by technicians to vary slightly (but not significantly) and it became difficult to discriminate between color pairs such as light (PG-104) and dark (PG-105) green or between red (PG-101) and yellow (PG-102).

There was no significant difference in the detectability of marks on dead versus live fish. Examining live fish for marks resulted in only slight handling mortality in the first 24 h: 0.69% (n = 1,743) on one occasion and 0.2% (n = 12,581) on another. Examining such small and sometimes poorly marked fish is extremely time-consuming. One person could check only between 75 and 101 live fish per hour.

In summary, despite the various difficulties encountered, we believe this method is preferable to alternative batch marking techniques available for use with small species.

Acknowledgments

We are grateful to J.A. Mathias, M.F. Curtis, D.W. Lint, J. Martin, M.E. Whittaker, M. Papst, and G. Curry for their generous assistance during this study. Financial support was provided by grants to the senior author from the Canadian Department of Fisheries and Oceans and the University of Winnipeg.

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Accepted 29 March 1982

Use of Self-feeders for Rearing Steelhead Trout at Dworshak National Fish Hatchery

From mid-November 1980 to mid-April 1981, Dworshak National Fish Hatchery, Ahsahka, Idaho, used the self-feeding method to rear steelhead trout (*Salmo gairdneri*). Resulting food-to-flesh conversions were compared to those obtained by hand-feeding during the same period.

Four single-pass ponds were equipped with Model 125 Babington Response Feeders (Fig. 1; Babington Enterprises, Hagerman, Idaho). Steelhead trout in the four self-fed ponds numbered 39,785, 33,953, 33,749, and 33,510 when the test began and mean fish weight was 34 g. Two test ponds used for comparison of results from hand-feeding contained 33,422 and 33,271 steelhead trout (average weight, 44 g).

Food used in the self-feeders was 8/64 Abernathy diet. Hand-feeding was with 1/8 Oregon Moist Pellet diet. Hand-feeding rates were based on guidelines described by Buterbaugh and Willoughby (1967). Water temperatures through the period ranged from 4.4 to 10°C (average, 5.8°C).

Conversion data for the test ponds are shown in Table 1. Overall, self-fed fish gained 17 g per fish, whereas the hand-fed group gained 8 g per fish. In view of the potential cost savings resulting from a change to the Abernathy diet from the Oregon moist pellets, and the more efficient food-to-flesh conversion, further testing of self-feeders is planned at the Dworshak hatchery. Additional testing will focus on the suitability of self-feeders for use in the two recirculating systems.

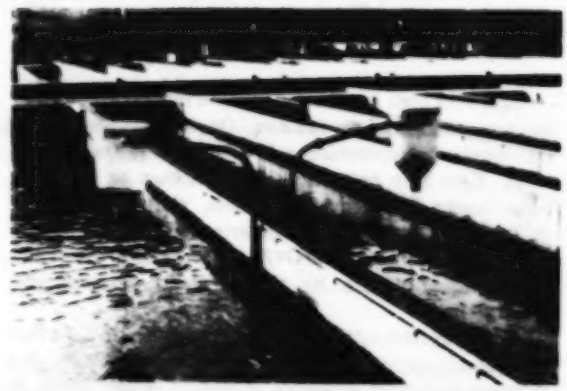

Fig. 1. Model 125 Babington Response Feeders mounted on pond wall.

Table 1. Conversion summary for self-fed and hand-fed groups of steelhead trout (mid-November 1980 to mid-April 1981).

Diet and delivery method	No. of fish (initial)	Weight gain (kg)	Food fed (kg)	Conversion
Self-fed	39,785	554	1,251	2.26
Abernathy	33,953	613	1,243	2.03
	33,749	377	954	2.53
	33,510	484	1,068	2.21
Summary	140,997	2,028	4,516	2.23
Hand-fed	33,422	221	1,036	4.69
Oregon moist pellet	33,271	280	1,141	4.08
Summary	66,693	501	2,177	4.35

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Accepted 29 March 1982

Toxicity and Residue Studies of Cultured Blue Shrimp Treated with the Algicide Cutrine-Plus

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ABSTRACT: Laboratory bioassays on the blue shrimp *(Penaeus stylirostris)* were conducted with Cutrine-Plus, a chelated copper algicide registered for use in fish hatcheries but not for shrimp culture facilities. The 24- and 96-h LC50's for blue shrimp were found to be 151.3 and 19.5 mg/L copper as Cutrine-Plus, respectively. Cutrine-Plus was found to be safe (i.e., produced no statistical mortality) at a use rate of 1.0 mg/L for a 24-h static exposure. The 24-h EC50 for gill melanization was 0.64 mg/L copper. In residue studies no accumulation of copper in whole-body tissue due to the treatments could be shown.

Algae have both positive and negative effects on pond and raceway shrimp culture facilities. On the positive side, in high-density shrimp culture tanks and raceways (Salser et al. 1978), algae have been shown to contribute to improved water quality by removing ammonia, nitrates, and other shrimp waste products, by supplying trace nutrients like ascorbic acid (Lightner et al. 1979; Magarelli et al. 1979), and by producing oxygen by photosynthesis (Supplee and Lightner 1976).

On the negative side, however, some species of algae that are typically present in shrimp culture ponds, tanks, and raceways have been implicated in reduced oxygen levels, in toxin production, and in attaching to the shrimp's gills and swimming appendages, thus causing reduced mobility and asphyxiation (Overstreet 1973; Johnson 1978). Algae which have been particularly troublesome in the shrimp culture facilities run by The University of Arizona in Puerto Penasco, Sonora, Mexico and on Oahu, Hawaii, are *Spirulina subsalsa*, three strains of *Schizothrix calciola*, the morphologically related but unpigmented *Leucothrix mucor* (presently classified with the bacteria), and numerous species of pennate diatoms.

Copper compounds have been widely used to control algal populations, but copper sulfate and copper chloride are rapidly precipitated in seawater (due to the higher concentrations of carbonates in seawater compared with fresh water), which results in a rapid reduction of algicidal effectiveness. The use of chelated copper algicides such as Cutrine-Plus (Applied Biochemists, Inc., Mequon, Wisconsin) eliminates this difficulty. Cutrine-Plus, a mono- and tri-ethanolamine copper chelate, has been registered by the U.S. Environmental Protection Agency (EPA) as a liquid algicide for use in fish hatcheries, but not (until February 1982) for use in shrimp or other types of aquaculture facilities. Aquatrine (Applied Biochemists), a product similar to its predecessor Cutrine-Plus, has been approved by the EPA for use in fish and shrimp aquaculture facilities (ponds, tanks, and raceways) as an algicide (EPA Reg. No. 8959-33).

Copper is toxic to aquatic animals at concentrations that depend on the animal species and the water chemistry (i.e., pH, hardness, and alkalinity). Using Cutrine, (the predecessor to Cutrine-Plus, 0.8 times as concentrated) Skea and Simonin (1979) reported a 96-h LC50 to fingerling largemouth bass (*Micropterus salmoides*) of 6.4 mg copper per liter at 21.1 °C (methyl orange alkalinity of 53 mg/L) and 0.21 mg/L at 18.3 °C (methyl orange alkalinity of 95 mg/L) for fathead minnows (*Pimephales promelas*). Johnson (1974), who tested copper sulfate with the marine shrimp (*Penaeus duorarum, P. stylirostris,* and *P. setiferus*), determined that copper sulfate could be used with juveniles of these species at levels of 0.5-1.0 mg/L as copper sulfate (alkalinity of 380 mg/L calcium carbonate.).

The objectives of the present study were to determine the toxicity of copper as Cutrine-Plus to juvenile blue shrimp (*P. stylirostris*), the extent of organ damage at various concentrations of Cutrine-Plus, and the amount of whole-body copper residue resulting from treatment with Cutrine-Plus, and, therefore, to obtain an indication of its safety for use in shrimp culture facilities.

Materials and Methods

Toxicity Studies

Bioassays with juvenile blue shrimp were conducted at the experimental shrimp culture facility of the Universities of

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Arizona and Sonora at Puerto Penasco, Sonora, Mexico. The facility and culture methods used were previously described (Salser et al. 1978). The seawater used (supplied from seawater wells) was 35-41‰ salinity and about 6,000 mg/L total hardness (as $CaCO_3$); pH was 7.4-8.4, and temperatures ranged from 23.5 to 24.5°C. Four static bioassays were conducted in 80-L fiberglass tanks supplied with constant aeration according to procedures outlined by the American Public Health Association et al. (1975). Throughout the bioassays, oxygen levels ranged from 6.0 to 8.0 mg/L as measured by oxygen electrode. In three acute toxicity experiments, each tank contained five shrimp (12-16 g) and treatments ranged from 12.5 to 100 mg/L copper with one replicate for a total of 10 shrimp per treatment (Table 1). The increments of the treatments were varied from experiment to experiment, depending on the results of the previous experiment. Shrimp were taken from rearing raceways at the facilities, immediately transferred to the test tanks, and acclimated for 2-4 h before exposure began. In an experiment to determine the relation between copper concentration and gill melanization, shrimp were treated (10 per treatment) with 0.2-4.0 mg/L copper as Cutrine-Plus for 96 h (Table 1). Gill melanization was measured by gross examination after 96 h and evaluated on the basis of the number of shrimp with melanized gills. The treatments were discontinued after 96 h and the shrimp were monitored for mortality for 1 week. Samples were selected for histological study on the basis of showing gill melanization, and preserved in Davidson's fixative (acetic acid, alcohol, formalin; Humason 1967:432), processed by using routine histological methods, and stained in hematoxylin and eosin.

The method of Litchfield and Wilcoxon (1949) was used in calculations of the 24- and 96-h LC50's (the dose that is lethal for 50% of the test animals) as well as the EC50 (the median effective concentration) based on gill melanization. Multiple linear regression was performed on the logarithm of the concentration versus the logarithm of time versus the percent mortality to determine the expected mortality level at normal usage rates.

Residue Studies

The residue study consisted of a pair of experiments in which a population of juvenile blue shrimp was given either a single treatment or multiple 6-h static treatments with Cutrine-Plus at a concentration of 0.25 mg/L copper. Four 2,400-L tanks were stocked with 200 shrimp (average, 12 g) from the raceways at the Puerto Penasco facility for the single-exposure exprimeni; two 2,400-L tanks were similarly stocked for the multiple exposure experiment. Seawater in the tanks (salinity, 35-36 ‰; temperature, 25 ± 2°C) was exchanged about 10 times per day, and flowed continuously except during treatments. Continuous aeration was supplied to keep the oxygen levels at 6.0 mg/L. Hardness and pH were within the values stated above.

In the single-treatment experiment, three shrimp were sampled at random for whole-body copper anaylsis at 6, 12, 24, 48, and 96 h after treatment. In the multiple-treatment experiment, shrimp were treated at 7-day intervals for 6 weeks and sampled (three shrimp per sample) 1 and 6 days after each treatment. Random untreated controls were also taken at each sampling interval in both experiments. Independent commercial laboratories analyzed the whole-body copper content by atomic absorption spectrometry, using standard tissue methods.

Table 1. Toxicity of Cutrine-Plus to juvenile blue shrimp: percent survival of shrimp after exposure to concentrations of 12.5-100 mg/L for 24 or 96 h, and percent with gill melanization after exposure for 96 h to 0.2-4.0 mg/L.

Study and concentration of Cutrine-Plus (mg/L)	Survival (%)	
	24 h	96 h
Survival study		
0 (control)	100	100
12.5	100	70
25.0	100	40
50.0	100	0
54.5	90	—
52.9	90	—
59.5	90	—
64.8	80	—
70.7	90	—
77.1	70	—
83.3	80	—
84.1	80	—
91.7	60	—
100.0	90	—
100.0	70	0
Gill melanization study		
0 (control)	—	0
0.2	—	0
0.5	—	30
1.0	—	100
2.0	—	80
4.0	—	100

Results

Toxicity Studies

The use of Cutrine-Plus produced 24- and 96-h LC50's, and a 96-h EC50 for gill melanization in juvenile blue shrimp, as summarized in Table 2. No mortality was observed during the 1-week monitoring period following the gill melanization experiment.

Examination for organ damage showed some melanization of the gills of juvenile blue shrimp exposed for 96 h to up to 4.0 mg/L copper as Curtrine-Plus. Damaged gills became melanized within 24 to 96 h (depending to some extent on increasing concentration, Table 1) following start of exposure, and histological examination showed scattered to numerous

Table 2. Calculated lethal and effective concentrations from results of toxicity experiments of Cutrine-Plus to juvenile blue shrimp.

Level	Concentration (mg/L)[a] 24 h	96 h
LC1	45.9 (29.9 - 79.4)	6.9 (3.2 - > 100)
LC50	151.3 (123.5 - 258.9)	19.5 (12.5 - 30.5)
EC1		0.2 (0.0 - 0.4)
EC50		0.6 (0.4 - 1.1)
EC99		2.4 (1.6 - > 100)

[a]Numbers in parentheses represent 95% confidence intervals.

focal necrotic, inflamed, and melanized lamellae. The epithelium of portions of the bases of the branchiostegal processes was also melanized. The 96-h median effective concentration for gill melanization was 0.64 mg/L copper as Cutrine-Plus. Gill melanization was rapidly resolved by the affected shrimp, and no mortalities occurred in this experiment at the highest treatment level (4.0 mg/L) during the experiment or post-exposure period. Gross and histological examinations of shrimp from this study showed that the gills and associated gill processes were the only damaged organs.

Residue Studies

The single treatment experiment in the residue study produced a mean whole-body copper content of 87.3 ± 17.1 mg/kg dry weight for the treated shrimp and 78.3 ± 18.6 mg/kg for the control group (Table 3). The overall mean for the treated shrimp in the multiple-treatment experiment was 100.2 ± 27.8 mg/kg dry weight and that of the control was 84.3 ± 19.2 mg/kg (Table 3). These values are not significantly different at the 0.05 level by analysis of variance.

Table 3. Mean whole-body copper levels of shrimp (three shrimp per sample) after 6-h treatments with Cutrine-Plus (0.25 mg/L copper).

Time after treatment	Treatment No.	Copper content (mg/kg dry weight) Control (Replicate) 1	2	Treated (Replicate) 1	2
Single 6-h treatment					
Hours					
0		40	94	88	56
6		104	74	111	94
12		73	76	88	83
24		79	80	78	102
48		89	103	61	69
96		53	74	76	111
Mean		78.3		87.3	
Range		40-104		56-111	
SD		±18.6		±17.1	
Multiple 6-h treatments					
Days					
—	0	40		88	
1	1	79		78	
6	1	100		81	
1	2	68		65	
6	2	95		70	
1	3	110		127	
6	3	76		80	
1	4	76		133	
6	4	82		89	
1	5	96		116	
6	5	83		138	
1	6	107		137	
Mean		84.3		100.2	
Range		40-110		65-138	
SD		±19.2		±27.8	

Discussion

The toxicity of Cutrine to fish given by Skea and Simonin (1979) is 3 to 100 times greater than those we found for juvenile penaeid shrimp. The results of the present work agree favorably with those of Johnson (1974), indicating that penaeid shrimp have a high tolerance for copper ethanolamine complexes. It is, however, apparent from the present studies that copper (as Cutrine-Plus) produces an apparently minor toxic effect on juvenile shrimp at concentrations below those required to kill the animals. From the data (Table 2), the effect produced (EC99; gill melanization) has little effect on mortality below concentrations of 3.2 mg/L (LC1 lower limit) with a 96-h exposure. Hence, concentrations in the range from 1.55 to 3.2 mg/L should produce grossly detectable gill melanization in 99% of a population while producing less than 1% mortality after an exposure of 96 h. Since only one experiment was performed for gill melanization, the 95% confidence interval for the EC99 was extremely wide and all doses tested in the toxicity studies fell within this interval. In our facilities, it has been found that a static exposure of 0.25-0.5 mg/L for 4 h is adequate for controlling algal populations. Multiple linear regression of the toxicity data shows that there would be no statistical mortality with these use rates, or with a 24-h exposure at 1.0 mg/L; with a 4-h exposure, 430 mg/L would be required to produce a 1% mortality.

In the present study, the findings for whole-body copper residues agree favorably with those of Lin et al. (1972), who found that copper residues in rainbow trout (*Salmo gairdneri*) were related neither to the length of exposure nor to the concentration of Cutrine. However, trout accumulate copper residues to an average of 1.1 times the pretreatment level. In contrast, penaeid shrimp showed such a wide range in their normal total body copper levels in the present study that no accumulation could be shown. This wide range may be the norm for Crustacea since Boone and Schoeffeniels (1979) reported a wide range of whole-body copper levels for the shore crab (*Carcinus maenus*).

On the basis of the present study, we conclude that Cutrine-Plus can be used in high density culture of penaeid shrimp for control of algae and *Leucothrix mucor* at levels found to be effective (0.1 mg Cu/L for up to 24 h, Lightner and Supplee 1976; 0.25-0.5 mg Cu/L for 4-6 h, Lightner 1977), with no mortality and little damage to the animals and without significant accumulation of copper in such treated, cultured shrimp.

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Accepted 30 April 1982.

Cost Analysis of Aquatic Weed Control: Fish Versus Chemicals in a Florida Lake

The grass carp (*Ctenopharyngodon idella*) has been researched extensively as a weed control agent, as evidenced by extensive literature on the subject (Smith and Shireman 1981). The use of grass carp for weed control is reported to be less costly than other methods and weeds are controlled for a longer time (Aliev 1963; Rottmann 1977). The use of grass carp is controversial, however, because of their ability to spawn in U.S. rivers and the possible environmental impact. Even though the grass carp has received this attention, little information is available concerning cost benefits that might result from the use of the grass carp. Data are presented in this paper concerning the actual costs of herbicides and grass carp for the management of hydrilla (*Hydrilla verticillata*) in a Florida lake.

Lake Baldwin (80 ha), in Orange County, Florida, is almost completely within the confines of the U.S. Navy Training Center at Orlando. The Navy is responsible for hydrilla treatment within the lake. Fingerling grass carp (1,999; 102-152 mm TL; $1 each) were first stocked by the Florida Game and Fresh Water Fish Commission during April 1975. Survival of these fish was poor (Colle et al. 1978) and a second stocking of grass carp (2,000; > 300 mm) was necessary. The cost of grass carp and hybrid grass carp (> 300 mm) was estimated at $3 and $5 per fish, respectively, which is their current market value. An additional $0.25 was added per fish to cover transportation and labor costs at stocking. The U.S. Navy provided costs of herbicide treatments. Hydout [Mono (N, N-dimethylalkylamine)] was applied for vegetation treatment according to label recommendations.

Costs for chemical treatments from calendar years 1973 through 1981 for Lake Baldwin, including labor, are presented in Table 1. From 1973 through 1975, chemical treatments were made according to hydrilla coverage, but hydrilla was not completely controlled in any of these years. The 1975 grass carp stocking was partly successful, even though mortality was high; hydrilla coverage remained below pre-stocking levels until 1978. A population estimate (Colle et

Table 1. Cost of vegetation treatment, Lake Baldwin, Florida, 1973 through 1981.

Year	Herbicide costs			Grass carp costs
	Materials	Labor	Total	
1973	$ 32,775	$1,955	$ 34,730	—
1974	40,648	2,226	42,874	—
1975	21,612	1,401	23,013	$1,999
1976	—	—	—	—
1977	4,061	753	4,814	—
1978	11,092	709	11,801	—
1979	—	—	—	$6,500
1980	—	—	—	—
1981	—	—	—	—
Totals	$110,188	$7,044	$117,232	$8,499

al. 1978) indicated that only 3 fish/ha remained in the lake after 2 years, a number insufficient to keep hydrilla under satisfactory control. About 20 ha of the lake were treated in 1978, which temporarily decreased hydrilla along the shoreline. In 1979, 2,000 additional grass carp were stocked; total stocking equaled 35 grass carp per hectare of hydrilla. From 1979 through 1981 (Table 1), chemical treatments were applied and hydrilla was completely removed from the lake during this period by the grass carp (Shireman and Maceina 1981).

The costs of herbicide treatments before the grass carp were stocked were considerable and control was temporary. When the 3 years before stocking grass carp (1973 through 1975) are compared with the years after stocking (1973 through 1981), the cost of treatment was decreased by $79,501 if the cost of the first grass carp stocking is included in the 1973 to 1975 category. Greater cost benefits would have accrued if larger grass carp had been stocked in 1975, because control would have been achieved sooner.

Hydrilla has become a serious aquatic weed problem in Florida. Haller (1979) estimated that 15,000 ha of hydrilla were treated in Florida during 1977 at a cost of about $9.1 million. If grass carp had been used alone or in conjunction with these treatments, considerable money could have been saved. For example, the cost of grass carp stocked at 35 fish/ha of hydrilla would have been $1.71 million. Grass carp treatments would also have been long-lasting, alleviating the problem for several years.

The use of hybrid grass carp (grass carp × bighead carp, *Aristichthys nobilis*) is being researched as a sterile replacement for the grass carp. Available data indicate that the fish do not consume vegetation as voraciously as grass carp; therefore, greater numbers are needed for stocking. One estimate (John Osborn, University of Central Florida, personal communication) places the stocking rate at three times that of grass carp. Hybrid stocking by the Florida Game and Fish Commission (Ware 1981) also indicates that greater numbers of hybrids are needed for vegetation control. In four lakes the lowest stocking rate used was 62 hybrids per hectare (I assume this stocking rate is for the entire lake and not per hectare of hydrilla). A reduction in hydrilla was not observed during the first year in this lake, and is not expected until the second growing season. Hybrids were successful where chemical treatments were used in conjunction with stocking. Stocking hybrids, therefore, will be more costly. As an example, if 3 times the grass carp stocking rate for hybrids had been used in Lake Baldwin, the 1979 stocking would have cost $31,500. It appears that if the hybrid is to be a useful replacement for grass carp, additional herbicides must be used before stocking to decrease plant biomass and, subsequently, the hybrid stocking rates. The advantage of using the two simultaneously should be more effective and longer term control.

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Accepted 29 March 1982

Induced Spawning and Culture of Bonytail Chub

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ABSTRACT: During 1981, bonytail chub (*Gila elegans*) were spawned and cultured at Willow Beach (Arizona) National Fish Hatchery. This species is listed as endangered by the U.S. Fish and Wildlife Service. Five females ovulated 18 to 20 h after intraperitoneal injections of 4 mg acetone-dried carp pituitary per kilogram of body weight. Eggs were manually stripped and artificially fertilized; mean fecundity was 25,090. Incubation periods were 99 to 174 h at water temperatures of 20-21 °C, 170 to 269 h at 16-17 °C, and 334 to 498 h at 12-13 °C. Survival of eggs was 90% at 20-21 °C, 55% at 16-17 °C, and 4% at 12-13 °C. At hatching, total length and weight of fry averaged 6.8 mm and 2.8 mg. Survival of swim-up fry was 98% at 20-21 °C, 96% at 16-17 °C, and 25% at 12-13 °C. Fry placed in raceways doubled in length by 28 days, and fingerlings were 42.9 to 54.3 mm in total length and weighed 714.1 to 1,326.8 mg 70 days after hatching.

Little is known about the biology of the bonytail chub (*Gila elegans*), a species of cyprinid endemic to the Colorado River basin. Once abundant throughout the upper and lower basins (Cope and Yarrow 1875), this species has experienced the most abrupt decline of any fish native to the mainstreams.

In the upper basin, the only bonytail chubs reported in recent years were from the Green River in Utah (Behnke and Benson 1980). Although the species is occasionally found in the reservoir areas of lakes Havasu and Mohave in the lower basin (Minckley 1973), it is considered in danger of extinction. The bonytail chub is listed as an endangered species by the States of Arizona, California, Nevada, Utah, and Wyoming (Deacon et al. 1979) and by the U.S. Department of the Interior (1980).

The purpose of the present study was to develop techniques for the spawning and culture of bonytail chub. Data regarding the first successful spawning of wild brood stock in captivity and culture of their progeny during 1981 at Willow Beach (Arizona) National Fish Hatchery are presented.

Methods

The brood stock consisted of six wild females and five wild males collected from Lake Mohave, near Cottonwood Cove, 30 April to 1 May 1979, 29 April to 3 May 1980, and 11-12 March 1981. Total lengths and weights of the females were 487-564 mm and 956-1,500 g; males were 454-480 mm long and weighed 728-870 g.

Ovulation was induced with injections of carp pituitary (Ball and Bacon 1954; Clemens and Sneed 1962). Acetone-dried carp pituitary (40 mg) was mixed with a solution of oxytetracycline hydrochloride (10 mL) and injected at the rate of 4 mg/kg body weight at 24-h intervals (Hamman 1981). Fish were anesthetized with tricaine methanesulfonate at 50-100 mg/L (Leitritz 1960) before intraperitoneal injections, and then placed in a holding tank (6.0 × 0.75 × 0.75 m) until they were manually stripped. Warm water (20-21 °C) flowed through the tank at the rate of 23 L/min. Natural spawning of bonytail chubs is believed to occur when the water temperature reaches 18 °C (Vanicek and Kramer 1969; U.S. Fish and Wildlife Service 1979).

When eggs could be expelled with slight pressure, a female was anesthetized and wiped dry before the eggs were stripped into a plastic pan. Milt from one male was added to the eggs, sperm diluent (Billard 1977) at 12,000 mg/L was added to activate the sperm, and eggs were stirred and washed for 45 min to prevent clumping. After water hardening (about 50 min), eggs were poured on screen trays (53 × 53 cm) covered with 1.4-mm-mesh hardware cloth and placed in four aluminum troughs (3.5 × 0.55 × 0.3 m) and slanted at a 30° angle into the incoming water. To determine incubation periods at different water temperatures, warm water (20-21 °C) entered two troughs, cool water (16-17 °C) entered one trough, and cold water (12-13 °C) entered the fourth trough, all at 7.5 L/min.

The day after the fry emerged, they were transferred to an outside recirculating system consisting of two concrete raceways (30.5 × 2.4 × 1.2 m) that were designed as B1 and B2. Water was pumped to the upper end of raceway B1 from the adjacent raceway (B2) by a ½-hp fresh-flo pump at 0.01 m^3/s, flowed to the lower end, and returned to B2 by gravity. The raceways were filled 1 week before spawning to obtain warmer water temperatures; small amounts of inorganic (ammonium phosphate) and organic (commercial trout starter) fertilizers were added to produce a plankton bloom. Ten days after the fertilizers were added, zooplankton (predominately cladocerans) began appearing.

Results

Males and females had fine tubercles on the head, opercular, and pectoral and pelvic fins when spawning began (4

Table 1. Summary of data for six spawning female bonytail chub of unknown age, May 1981, at Willow Beach (Arizona) National Fish Hatchery.

Fish		Eggs	
Total length (mm)	Weight (g)	Fecundity (No. of eggs)	Fertilization (%)
533[a]	1,280	0	0
487	1,160	27,300	97
492	1,065	22,100	94
564	1,500	32,500	95
518	1,150	37,700	91
511	956	5,850	98

[a]Fish received three injections.

May 1981, water temperature 20 °C). Breeding males also had a light orange coloration at the base of the pectoral and pelvic fins; breeding colors were not present on the females. All males were producing seminal plasma with active sperm; however, no sex products could be expressed from any female until after hormonal injections.

Five females ovulated 18 to 20 h after the first injection; one female failed to ovulate after receiving three injections. Egg diameters ranged from 2.0 to 2.3 mm (mean, 2.2 mm); volumetric measurements varied from 103 to 151 eggs/mL (mean, 130 eggs/mL). Total fecundity was 125,450 (mean, 25,090/female) or 21,514 eggs/kg of body weight (Table 1).

Incubation and Hatching

Eggs placed in the 20-21 °C water began hatching at 99 h and were completely hatched by 174 h; total length of newly hatched fry averaged 6.8 mm (Table 2). During the incubation period at 20-21 °C, dissolved oxygen ranged from 9.25 to 9.5 mg/L, ammonia nitrogen from 0.03 to 0.04 mg/L, carbon dioxide from 2.77 to 2.95 mg/L, pH from 8.0 to 8.05 units, and water hardness from 325 to 355 mg/L. Eggs placed in the cool water (16-17 °C) began hatching at 170 h and hatching was completed by 269 h; newly hatched fry averaged 6.8 mm in total length (Table 2). Eggs placed in the cold water (12-13 °C) began hatching at 334 h and were finished by 498 h; total length of these newly hatched fry also averaged 6.8 mm (Table 2).

Culture

When fry began swimming to the surface, 48 to 120 h after hatching, 26,438 were placed in raceways B1 and B2; the remaining 80,000 fry were transferred to Dexter National Fish Hatchery in New Mexico.

During the first 14 days the fry were very active and fed near the surface. These fish (mean, 9.7 mm TL) swam in schools, but by day 21 the fry (mean, 11.5 mm TL) were scattered throughout the culture system. This behavior was believed to be due to the decline in zooplankton, which also occurred by the third week.

Table 2. Summary of data from incubating eggs of bonytail chub at three different water temperatures.

	Water temperature (°C)			
Incubation data	20-21	20-21	16-17	12-13
Number of eggs	80,600	37,700	5,850[a]	1,300
First cleavage (h)	3	3	4	5
Embryo forming (h)	22	24	29	52
Embryo developed (h)	68	69	101	162
Pigmented eyes (h)	76	79	133	222
Hatching began (h)	99	107	170	334
Hatching ended (h)	165	174	269	498
Percent hatch (%)	91	88	55	4
Number hatched	73,346	33,176	1,787	52
Age of swim-up fry (h)	48	48	72	120
Percent swim-up	98	99	96	25
Number of fry	71,879	32,844	1,715	13

[a]A total of 2,600 eggs were transferred to Utah State University at Logan.

Fry were fed a supplemental diet of commercial trout starter beginning on day 21. They were fed 4 to 5 times daily (0.25 to 0.50 kg of feed per day). By day 28, most fish (mean 18.1 mm TL) readily accepted the prepared diet. Schooling behavior was observed again on day 28 and continued through day 70.

Throughout the present study, continuous water temperature recordings were made and carbon dioxide, ammonia nitrogen, dissolved oxygen, and pH were analyzed each week to monitor water quality during culture. Water temperature varied from 12.8 to 24.4 °C, carbon dioxide from 1.8 to 4.4 mg/L, ammonia nitrogen from 0.01 to 0.81 mg/L, dissolved oxygen from 7.6 to 10.0 mg/L, and pH from 7.8 to 9.0 units.

On day 42 (when mean fry total length was 31.1 mm), *Icthophthirus multifilis* was microscopically identified on two dead fish. To prevent further losses, multiple treatments (three treatments on alternate days) of formalin at 15 mg/L (Allison 1957) were used. By day 49, the parasite was believed to be eliminated; however, weekly prophylactic treatments were continued to prevent further outbreaks.

Samples of fish were collected each week to determine growth (Table 3). On day 70, a total of 18,775 fingerlings (mean 49.5 mm and 974.6 mg) were removed from raceways B1 and B2; survival rate was 71%. The fish had increased 7-fold in total length and 348-fold in weight during 70 days.

Discussion

Based on the collection of gravid females, ripe males, and the appearance of juvenile fish in the upper Green River, Vanicek and Kramer (1969) concluded that bonytail chubs apparently spawn in June and July when the water temperature reaches 18 °C; however, natural spawning of this species has been observed in Lake Mohave during May (Jonez and Sumner 1954). During the present study, spawning occurred in May at water temperatures of 20-21 °C. Based on the three different egg incubation temperatures used in this study

Table 3. Total lengths and weights of larval bonytail chub cultured in a recirculating system at Willow Beach (Arizona) National Fish Hatchery, 1981.

Age (days)	Total length (mm)		Weight (mg)	
	Mean[a]	Range	Mean[a]	Range
0[b]	6.8	6.7- 6.9	2.8	2.3- 3.4
7	8.2	7.3- 9.1	3.6	2.8- 4.5
14	9.7	8.4-12.6	6.4	4.6- 14.4
21	11.5	10.2-14.1	8.7	5.7- 16.8
28	18.1	16.3-20.1	48.0	33.1- 70.7
35	20.2	19.1-21.6	76.4	62.8- 89.0
42	31.1	26.7-35.9	278.2	179.4- 397.3
49	36.1	30.6-43.9	448.7	253.3- 746.2
56	38.8	33.3-46.7	692.8	341.8- 839.9
63	43.6	36.7-47.8	740.7	466.1- 910.3
70	49.5	42.9-54.2	974.6	714.1-1,326.8

[a]Mean total length and weight of 10 fish per sample.
[b]Age of newly hatched larvae.

(Table 2), the optimum temperature for reproduction is believed to be 20-21 °C.

The decline of the bonytail chub has been correlated with the construction of dams which created cold downstream water temperatures, reductions in water flow, and loss of habitat (Joseph et al. 1977; Smith et al. 1979). The 96% mortality of eggs incubated at cold water temperatures supports this theory of dam-related decline. Another reason for the decline of the bonytail chub is the possibility of hybridization between the roundtail chub *(Gila robusta)* and humpback chub *(Gila cypha)* in the upper Colorado River (Holden and Stalnaker 1970; Minckley 1973). It is apparent that all three species are capable of hybridizing, because F1 generations of the bonytail X roundtail chub and bonytail X humpback chub have been produced in a hatchery (Hamman 1981).

The future of the bonytail chub may depend on hatchery propagation to help restore the species. In addition to providing specimens for restocking of selected former habitats and supplementing current populations, these culture efforts provide an opportunity to study the fish under hatchery conditions. Based on captive and cultured specimens, much is being learned about their reproduction, early life history, and preferences. This information may be necessary to insure the survival of the species.

Acknowledgments

I thank Lyle Miller, Peter Carboni, Joe Saenz, and the staff at Willow Beach National Fish Hatchery for assistance during this study, and Theophilus Inslee, Buddy Jensen, J. Mayo Martin, John Mazuranich, Joseph McCraren, William Miller, and Robert Piper for reviewing the manuscript.

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Accepted 2 April 1982

Modified Procedure for Aging Walleyes by Using Dorsal Spine Sections

The accuracy of age determination of walleyes (*Stizostedion vitreum vitreum*) made by using scales has been subject to some question, especially in older fish (Olson 1980). The use of bony structures (otoliths, vertebrae, branchiostegal rays, opercles, fin sections) offers considerably more accuracy, but generally requires sacrificing the fish. The use of dorsal spines has provided investigators with greater accuracy in age determination of walleyes without killing fish (Campbell and Babaluk 1979). Present methods require a specialized device to section spines (Campbell and Babaluk 1979) along with spine preparation techniques generally considered too laborious for field procedures. Attempts to simplify these methods also presented problems — sectioning of unmounted spines often resulted in shattering, or loss of the spine section as it was thrown from the cutting edge. The present note describes an effective and inexpensive modifiction of methods for processing spines that is convenient for use in everyday management.

In the field, the second dorsal spine is removed as close to the body of the fish as possible with a pair of 10.2-cm (4-in) side-cutting pliers and placed in appropriately labeled sample envelopes. Extraneous fleshy material on spines is later removed in the laboratory by placing individual spine samples in jars containing bleach for several minutes.

Spine samples are mounted in clear casting resin, using wooden molds lined with parafilm. We used a mold with a trough of 8 × 8 mm (5/16 in.) and 51 cm (20 in.) long. In a mold of this size 15-20 spines can be mounted in one cast. When large numbers of spines are to be mounted, the use of several molds greatly speeds up the process. After parafilm has been laid in the mold, a base of about 3 mm (⅛ in.) of prepared casting resin is poured. Once the resin begins to gel, spine samples (trimmed of distal portions) are placed in sequence on the base, and an additional layer of casting resin is added to embed the spines. Hardening time varies, depending on temperature and humidity. Sample numbers are transferred onto the hardened spine cast, the parafilm is peeled away, and spines are ready to be sectioned.

Spine sectioning is done with a hand drill fitted with a Dremel saw blade #406 (Fig. 1). The spine cast is held in place between two grooved wooden blocks and secured by two bolts and wing nuts. The thickness of spine sections is determined by turning a threaded rod that fits through the spine cast holder into a nut secured in an adjacent block. A nut fastened to the rod on each side of the spine cast holder causes the spine cast and holder to move when the rod is turned. This rig is mounted on a platform with wooden runners which guides the spine cast as it is sectioned. Two or three sections of each spine (0.7-1.6 mm; 1/32-1/16 in.) are cut as near to the spine base as possible.

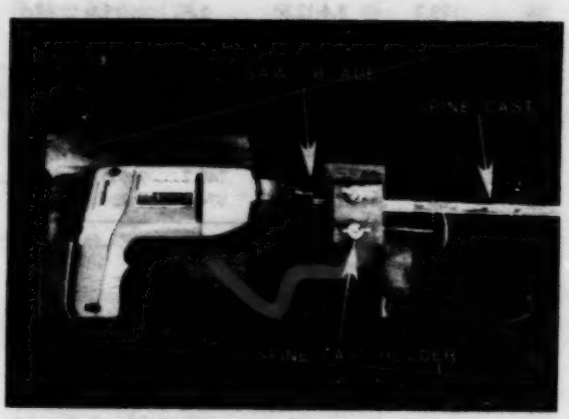

Fig 1. Sectioning device for walleye dorsal spines.

Spine sections are placed on a glass slide with mineral oil (clearing agent) and viewed at 50×, using a compound microscope and reflected light. This procedure enables adjustment of variable light intensities from different angles for more accuracy in aging. After aging, spine sections can be wiped clean and stored in sample envelopes for an indefinite period.

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Accepted 2 April 1982

Plasma Corticosteroids and Chlorides in Striped Bass Exposed to Tricaine Methanesulfonate, Quinaldine, Etomidate, and Salt

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ABSTRACT: Plasma chloride and corticosteroid concentrations were measured in yearling striped bass (*Morone saxatilis*) exposed to 25 mg/L tricaine methanesulfonate, 2.5 mg/L quinaldine, or 0.1 mg/L etomidate (an experimental drug), alone and in combination with 10 g/L salt (NaCl). Plasma chloride levels were unaffected in all treatments during a 15-min exposure and during a 10-min period of close confinement in a dipnet. Plasma corticosteroids increased in fish exposed to salt alone and in fish exposed to tricaine methanesulfonate or quinaldine, either alone or in combination with salt. Etomidate alone or combined with salt limited the increase in plasma corticosteroids during exposure and confinement. Etomidate appears to be a useful drug for suppressing physiological changes during the handling and transportation of striped bass.

The stress of handling and transportation of striped bass (*Morone saxatilis*) frequently results in high mortality, either during capture, loading, and transportation or several days after handling. The death of fish is believed to be due to at least two factors: (1) osmoregulatory dysfunction, characterized by reduced plasma electrolyte levels (Wedemeyer 1972); and (2) epizootics resulting from lowered disease resistance (Wedemeyer 1970). This lowered resistance may result from suppression of the immunological system; the result may be a decrease in the inflammatory response accompanying an elevation of corticosteroid hormones during stressful situations (Mazeaud et al. 1977).

The use of low concentrations of anesthetics (Smith 1978) and the use of salt (NaCl; McCraren and Millard 1978) in the water have been found to increase survival when fish are transported. Anesthetics reduce locomotor activity and metabolic rate (McFarland 1960) and salt is believed to reduce osmoregulatory dysfunction (Wedemeyer 1972). Tricaine methanesulfonate, quinaldine sulfate, and salt have been used in the transport of striped bass (Bonn et al. 1976). Etomidate {R-(+)-ethyl-l(l-phenylethyl)-1H-imidazole-5-carboxylate sulfate}, an experimental drug new to aquaculturists (Fragen et al. 1976), has been found to be an effective and well tolerated anesthetic in warmwater fish (J. Plumb, Auburn University, Auburn, Alabama, personal communication).

The objective of the present study was to quantitate corticosteroid and chloride levels in the plasma of striped bass exposed to anesthetics (tricaine methanesulfonate, quinaldine, and etomidate) and to salt. Treatments included exposure of the fish to each anesthetic alone and in combination with sodium chloride both before and after fish were stressed by confinement in a dipnet to simulate handling. Plasma corticosteroid levels were used as an indicator of the stress response, and plasma chloride levels were used as an indicator of osmoregulatory dysfunction.

Materials and Methods

Yearling striped bass (approximate average length and weight, 200 mm and 80 g) were held in nine 300-L tanks supplied with 24 L/min of aerated well water (21°C, 105 mg/L hardness, 105 mg/L alkalinity, pH 7.66). All tanks were equipped with spargers to supply continuous aeration. Fish were fed floating pellets, ad libitum, containing 38% protein. Food was withheld for 24 h before the beginning of a test.

All fish were quickly anesthetized in 200 mg/L tricaine methanesulfonate and were bled within 3 min. Exposure to this concentration of anesthetic resulted in the total loss of reflex activity in about 15 s and has been found to eliminate changes in corticosteroid hormones during at least the first 5 min of sampling (K.B. Davis, unpublished data). Blood samples were taken with a syringe from the caudal vessels in the hemal arch or with capillary tubes after the caudal peduncle was severed. Ammonium heparin was used as an anticoagulant and the plasma was stored frozen at −18 to −20°C. We determined plasma corticosteroid hormone levels in 25- or 50-μL samples by the competitive protein-binding method of Murphy (1967), using chicken plasma as the transcortin source (Fagerlund 1970). This method

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measures both cortisol and cortisone, and is reported as total plasma corticosteroids. Plasma chloride concentrations were measured by automatic amperometric titration with a chloridometer. Data were analyzed by a one-way analysis of variance, followed by Duncan's multiple range test. A probability level of 0.05 was considered significant.

Tricaine methanesulfonate, quinaldine, and etomidate were tested in concentrations selected to sedate fish. Sedated fish remained capable of maintaining normal body position and retained the ability to swim normally, but this activity was reduced. The sodium chloride level was chosen to be approximately isotonic and to prevent hypochloremia. We sampled 10 fish before beginning the experimental exposure to provide an undisturbed control (Fig. 1). Groups of 9 to 12 fish per tank were exposed to the chemicals by turning off the water supply and adding the appropriate amount of the compound. A group of eight fish were exposed only to fresh water after the water flow was stopped; these fish served as experimental freshwater controls. Blood samples were taken from about half of the group of fish in each tank after a 15-min exposure to fresh water, 25 mg/L tricaine methanesulfonate, 2.5 mg/L quinaldine, 0.1 mg/L etomidate, or 10 g/L sodium chloride, or to each anesthetic in combination with 10 g/L sodium chloride. The remaining fish were rapidly confined in a shallow dipnet and held in the net just below the surface of the water for an additional 10 min and then bled. This design allowed testing for changes in plasma corticosteroid and chloride levels after a standard 15-min exposure to the test chemical and after exposure to the stress of confinement sufficient to disrupt normal body position and movement.

Results and Discussion

Undisturbed striped bass had plasma corticosteroid concentrations (μg/100mL) of 2.99 ± 0.71 ($\bar{X}$ ± SE). Levels in freshwater control fish increased to 5.29 ± 0.87 after the first 15 min and increased further to 28.0 ± 2.08 after the 10-min confinement in a net (Fig. 1).

Plasma corticosteroid levels increased significantly in fish exposed 15 min to tricaine methanesulfonate, to salt, or to salt combined with tricaine methanesulfonate or quinaldine (Fig. 1). Exposure to quinaldine for 15 min also elevated the hormone levels, but the increase was not significantly different from that in freshwater controls. Corticosteroid concentrations in fish exposed to etomidate alone or combined with salt did not differ from those in freshwater control groups. Confinement for 10 min in a net after the 15-min exposure significantly elevated corticosteroid levels in all groups except those exposed to etomidate. Etomidate alone or in combination with salt markedly suppressed the elevation of plasma corticosteroids, whereas significant elevation occurred in stressed fish exposed only to salt.

Hypochloremia frequently develops when freshwater fish are handled, and the time at which chloride levels decrease varies with the species and with water quality (Wedemeyer 1972). However, plasma chloride levels were not significantly different from controls (118.8 ± 5.2 meq/L, $\bar{X}$ ± SE) in any

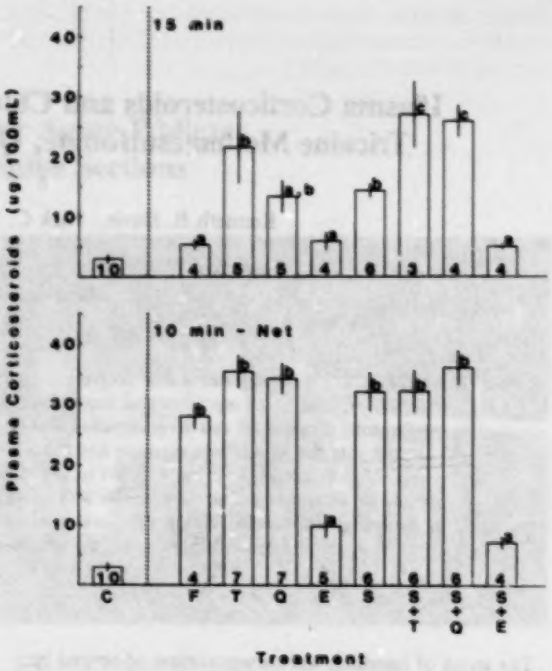

Fig. 1. Plasma corticosteroid concentrations (mean ± SE) in undisturbed striped bass controls (C), in fish exposed for 15 min to sedating concentrations of anesthetics and to salt (upper panel), and in fish exposed to anesthetics and salt and confined for 10 min in a dipnet (lower panel). F, freshwater controls; T, tricaine methanesulfonate; Q, quinaldine; E, etomidate; and S, salt. Number of fish is shown at the base of each column. Similar letters in each panel represent similar subsets at the 0.05 level by Duncan's multiple range test. Data represented in upper and lower panels were not compared; undisturbed controls included for reference only.

of the treatments. Wedemeyer (1972) demonstrated the immediate development of hypochloremia in salmonids after their netting and transfer to soft water. However, in a study of handling and transportation stress in hybrid striped bass Tomasso et al. (1980) reported decreased plasma chloride 24 h after handling. Consequently the absence of hypochloremia in our study may have been due to the short term of the experiment.

Elevation of corticosteroid hormone levels in response to a variety of environmental stressors is a well-known response in fish (Mazeaud et al. 1977; Specker and Schreck 1980). Such increases, particularly if prolonged, are considered to be detrimental to the long-term survival of the fish due to a suppression of the inflammatory response, decreased concentration of circulating leucocytes, and suppressed leucocyte migration and phagocytic activity (Grant 1967; Wedemeyer 1970). The data presented here suggest that etomidate might more effectively limit corticosteroid increases during handling and transportation of striped bass than would either tricaine methanesulfonate or quinaldine. Our study did not

address the long-term consequences resulting from exposures to these compounds. Further studies are needed to determine the possible delayed or long-term effects.

Salt has been shown to protect hybrid striped bass against short-term and long-term hypochloremia (Tomasso et al. 1980); however, in our study salt caused an increase in plasma corticosteroid hormone levels. We were unable to determine the possible positive or negative effects of corticosteroid elevations on striped bass survival. We expect etomidate in combination with salt to limit the corticosteroid hormone response and thus to increase both the short- and long-term survival of striped bass after handling and transportation.

Acknowledgments

We thank D. Amend of Tavolek, Inc., Redmond, Washington, for supplying the etomidate used in this study, and D. Amend, J. Geiger, B. Simco, K. Randolph, and G. Wedemeyer for reviewing the manuscript.

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Accepted 29 March 1982

Effects of Tilapia Densities on Growth of Channel Catfish

Monoculture, the production of a single species of fish in a system, is the major method of culturing fish in the United States and channel catfish (*Ictalurus punctatus*) is the primary warmwater species cultured. Because of success in catfish culture, studies have recently been done on culturing catfish with other species for the production of food fish (Buck 1977). The culture of two or more species together (polyculture) has been conducted successfully in many countries.

Tilapia and various species of carp (*Cyprinus carpio* — common, *Hypophthalmichthys molitrix* — silver, *Aristichthys nobilis* — bighead) have been polycultured in ponds with supplemental feeding, most notably in Israel (Buck 1977). Of the tilapias, *T. aurea* is the most commonly cultured in Israel (Brown 1977) and has worked well in polyculture because it occupies a different niche and uses different foods. In pond polyculture, however, their food habits are similar to those of channel catfish, except that as much as 62% of their diet may consist of pelleted feed and catfish feces (Smith 1973; Williamson and Smitherman 1975). Their reduced water quality requirements and the diversity of foods consumed by *T. aurea* make them a suitable choice for polyculture with channel catfish. Few studies have been done on polyculture of catfish and tilapia in raceways or other high density, flow-through systems (Allen and Carter 1976); the lack of information for such systems and the compatability of *T. aurea* and channel catfish in ponds prompted the present investigation. Our objectives were to determine the feasibility of high-density polyculture of tilapia and channel catfish in a flow-through system, to assess the effects of various stocking densities on growth and condition of catfish, and to

determine the practicality of the method by monitoring selected water quality variables.

In the present study, we used 12 fiberglass tanks 100 × 40 cm and 55 cm high, housed on a floating barge at Norris Dam Marina, Norris Reservoir, Tennessee. Surface-draining standpipes, 25.4 mm (ID), were used to maintain a constant water volume of 180 L in all tanks. A 0.5-hp continuous-duty well pump supplied lake water from about 2 m below the surface at a rate of about 47 L/min.

Two headtanks (105 × 44 cm and 50 cm high) were used to provide an even flow to the culture tanks. Each was fitted with polyvinyl chloride (PVC) pipe and valves to control water flow into each tank. The original flow rate of 1.5 L/min (2-h exchange rate per tank) was spilled onto the tank surface through the cover screen. After 30 days the flow was adjusted to 2.0 L/min (1.5-h exchange rate per tank) and the flow was discharged at the bottom of each tank. This change was made in an effort to prevent low concentrations of dissolved oxygen (DO) which inhibit fish growth. A 12-V electrical back-up system was used to maintain continuous water flow in the event of a power failure.

On 5 and 6 July 1979, *T. aurea* were obtained from Southern Fish Culturists, Leesburg, Florida, and were stocked in nine tanks at three densities, each replicated three times. These selected densities (3.0, 6.0, and 12.0 g/L) correspond to stocking rates of 270, 540, and 1,080 g/L per minute of flow, respectively. Channel catfish were obtained from a local fish farmer and were stocked 6-12 July in all 12 tanks at a rate of 12 g/L. Three tanks were stocked with catfish only as controls. All tanks were randomly selected for each replicate and covered with fiberglass screen to prevent fish from jumping out of the tanks. The average weights at stocking of tilapia and catfish for each treatment are summarized in Table 1.

On day 6 and every 14 days thereafter, gross weights of tilapia and catfish in each tank were recorded. A formalin treatment of 100 mg/L was applied to each tank after weighings to prevent external disease problems from handling. All fish were individually weighed and measured on day 90.

Fish were fed Purina floating catfish ration (36% protein) once daily except on Sundays and the days when fish were weighed. The feeding rate was 4% of the actual weight of the catfish in each tank and the amount of feed was adjusted every 14 days, when gross weights were taken.

Weekly water quality samples were taken in the morning from each culture tank and head tank. All samples were frozen and analyzed at the end of data collection. Total nitrogen due to ammonia ($N\text{-}NH_3$), nitrogen due to nitrate plus nitrite ($N\text{-}NO_2 + NO_3$), and pH were determined with a Technichon Auto Analyzer. Water temperatures and DO concentrations were recorded twice each week.

The change in the average weight of fish, condition factors, DO concentrations, pH, $N\text{-}NH_3$, and $N\text{-}NO_2 + NO_3$, were compared by using a nested one-way analysis of variance to detect significant differences between treatments. Duncan's multiple range test was applied when significant differences

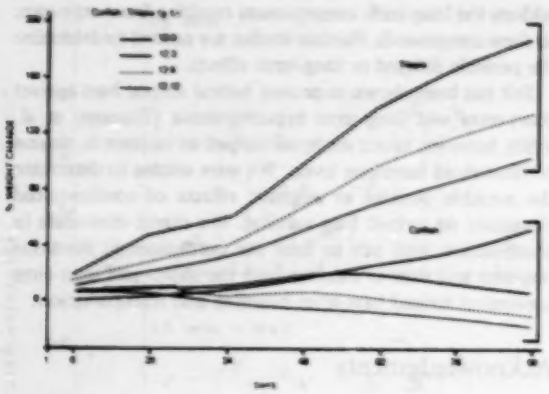

Fig. 1. Relative change in weight over time for tilapia and catfish.

existed between treatment means. Also, weight change, DO, stocking density, $N\text{-}NH_3$, and $N\text{-}NO_2 + NO_3$ were examined by regression and correlation analyses. A probability level of $P = 0.05$ was used in determining significance for all analyses.

As the stocking density of tilapia was increased, the growth of catfish decreased significantly in all polyculture treatments in comparison with the growth of monocultured catfish (Table 1). Monocultured catfish (12 g/L) gained about 49% of their initial body weight, whereas catfish stocked at an equal density but with 12 g/L of tilapia lost about 22% of their original body weight. The increased competition with increased tilapia density was also evident from the percent weight changes of the catfish. Catfish stocked with 12 g/L tilapia lost weight steadily for most of the 90 days; when stocked with 6 g/L tilapia the catfish lost weight slowly over the 90 days, and with 3 g/L tilapia they did not lose weight until after 45 days (Fig. 1). Tilapia in all treatments gained weight steadily over the 90 days.

Growth of tilapia was significantly less only at the highest stocking density (Table 1). In all polyculture treatments the tilapia had a much larger percent weight gain than the monocultured catfish (Fig. 1). Because of the more rapid growth of the tilapia, the weight ratios in the treatments had changed drastically from the initial stocking weight ratios after 90 days. As the density of tilapia increased, so did the degree of competition. We observed that tilapia stocked at 12 g/L were much more aggressive at feeding time than those stocked at lesser densities. Tilapia at 12 g/L fed before the catfish and waited for food at the surface whenever the tank cover was removed. We also observed tilapia biting catfish during feeding, which apparently limited the amount of food consumed by the catfish.

Condition factors of channel catfish were significantly different for each treatment, decreasing as the density of tilapia increased (Table 1). Condition factor was also an indication of the increased competition between the species as

Table 1. Comparison of changes in mean weights and condition of channel catfish and tilapia after 90 days. The letter after a mean denotes its rank in comparison to other means at 0.05 level of probability.

Stocking density (g/L) Catfish: Tilapia	Catfish			Tilapia		
	Initial weight (g)	Weight change (%)	Change in condition	Initial weight (g)	Weight change (%)	Change in condition
12:0 (control)	33.5	48.8 w	0.04 w	—	—	—
12:3	42.2	1.9 x	−0.07 x	31.2	186.7 w	0.28 w
12:6	42.8	−14.0 y	−0.10 y	43.2	133.1 w	0.26 w
12:12	44.9	−22.3 z	−0.18 z	35.5	101.3 x	0.18 x

density increased. Only tilapia at 12 g/L had a condition factor significantly lower than that of the others, due partly to the reduced amount of feed offered toward the end of the 90 days, since the ration was adjusted on the basis of the decreasing weight of catfish. Even so, the tilapia gained weight.

Correlation of species densities and growth of catfish resulted in a significant negative relation (Pearson correlation = −0.74). Although the analysis does not establish a definite cause-and-effect relation, it does suggest that as density increases the weight gain decreases.

Other than possible effects of low DO concentrations, water quality at the different stocking densities was not significantly different. Ammonia concentrations and nitrate-nitrite concentrations were not significantly correlated with density. Nitrate-nitrite levels were low enough not to have been toxic or to have had an inhibitory effect on growth. Since DO concentrations of 36% air saturation or less will inhibit growth (Andrews et al. 1973), there were times when the DO was low enough in the polyculture treatments to possibly have had an effect on growth (1.7-2.5 mg/L). Correlation analysis revealed a significant negative relation between DO concentrations and density (Pearson correlation = −0.83). A similar relation was found for stocking density and dissolved oxygen by Andrews et al. (1971). Thus, we conclude that stocking densities might influence growth through decreased oxygen concentrations as density increases. Water temperatures ranged from 20 to 29°C and remained above 23°C until day 86.

On day 16 a total of four deaths occurred in two of the monoculture tanks. Continued mortalities of catfish in the one tank revealed a disease problem, later diagnosed at Auburn University as *Edwardsiella tarda*. Terramycin-coated feed was used in treatment until laboratory tests showed the bacteria were resistant to terramycin. Treatment was then changed to Furacin-coated feed, which was given to all fish. All catfish in one monoculture tank were lost. No bacterial or parasitic involvement was observed among the tilapia. During the present study, tilapia reproduced in five of the tanks with stocking densities of 3 and 6 g/L of tilapia. The use of energy for reproduction and mouth-brooding of eggs and yolk-sac larvae by females might have had an effect on growth rates also. However, these effects were considered negligible because reproduction was limited.

From this study, we concluded that stocking *T. aurea* at densities equal to or greater than 25% of the weight of stocked catfish in a flow-through system with a high density of catfish would not be practical. Because of competition with the tilapia, decreased growth and condition of catfish would result in an unmarketable fish. A feeding rate greater than that used would probably not be effective in producing favorable growth, since the aggressive behavior (biting) of tilapia apparenty intimidated the catfish during feeding. In the present study, competition was the primary limiting factor, with lowered DO concentrations (due to stocking densities) as a contributing factor.

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Accepted 30 March 1982

Studies on the Mineral Requirements of the Adult American Lobster

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ABSTRACT: In a series of laboratory and field studies, the effects of dietary mineral supplements were tested on adult lobsters (*Homarus americanus*). Both semipurified diets and diets formulated from commercial ingredients were used. Results of these studies indicated that the optimum ratio of calcium and phosphorus (Ca:P) for adult lobsters was about 1:1, which agrees with optimum levels determined previously for juvenile lobsters. Results also indicated that added mineral supplements are of no particular benefit to adult lobsters in diets formulated for commercial use in pounds, as long as the ash present in the commercial ingredients has a Ca:P ratio near 1:1.

Each year in Maine and the Canadian Maritime provinces, millions of kilograms of lobsters (*Homarus americanus*) are held in high-density confinement in pounds for as long as 6 months. A lobster pound is very similar to a feed lot. Lobsters that are placed in a pound usually have molted recently and their muscle mass is small in comparison with the mass of their new, large, soft shell. Lobsters are fed during the holding period to increase muscle mass, harden the shell, and reduce cannibalism. Herring scrap from the sardine industry has been the traditional diet for lobsters in pounds. However, a constant supply of good quality herring is not always available and availability may be further reduced by the imposition of fishing quotas. While working on the formulation of an inexpensive, readily available diet for use at commercial pounds, we completed a series of studies to determine the benefits of mineral supplements in diets prepared for field use.

Materials and Methods

Dietary trials were conducted at two facilities. (1) Field trials were conducted at the Tidal Falls Lobster Pound, Hancock, Maine. At these facilities, lobsters on each test diet were placed in individual wooden storage crates and held in a floating lobster car. (2) Trials in our laboratory were conducted in wooden tanks covered with fiberglass with closed circulating systems and artificial (Instant Ocean) seawater. Salinity was held at 30 ± 3‰, and temperature was held at 18 ± 1°C. Tanks were equipped with a layered filter of synthetic sponge, activated charcoal, and calcium carbonate. Use of these filters maintained a proper pH level (7.0) and nitrifying bacteria in the sponges kept ammonia at a minimum level (< 1 mg/L).

A standard *t*-test or analysis of variance was used to test for significant difference between treatment means in each study. The level of significance is accepted as being $P < 0.05$ (unless otherwise stated).

Study 1. The first study was conducted to determine the effect of varying the ratio of calcium and phosphorus (Ca:P) in the diet of American lobsters under field conditions. Semipurified ingredients were used in this study to insure that the Ca:P ratios would be exact. The Ca:P ratios tested were 2:1, 1:1, and 0.12:1. Diet ingredients (composition given in Table 1) were mixed with water to form a dough for pelleting in a Hobart extruder. Moist pellets were dried at 40°C and stored as dried pellets in plastic bags until ready for use.

Table 1. Percentage composition of semipurified diets with different Ca:P ratios.

	Diet number and Ca:P ratio		
	1	2	3
Ingredient	0.12:1	1:1	2:1
Casein	14.0	14.0	14.0
Fish meal	15.0	15.0	15.0
Albumin	1.4	1.4	1.4
Yeast	10.0	10.0	10.0
Wheat gluten	9.0	9.0	9.0
Corn starch	24.0	24.0	24.0
Alphacel	11.4	8.3	4.9
Cod liver oil	2.0	2.0	2.0
Vitamin mix formula	2.0	2.0	2.0
Cholesterol	0.5	0.5	0.5
Choline chloride	0.7	0.7	0.7
Mineral mix[a]	10.0	10.0	10.0
Calcium carbonate	0.0	3.1	6.5
Ca:P ratio	0.12	1.00	2.00

[a]ICN Nutritional Biochemicals, Cleveland, Ohio (Bernhart and Tomarelli 1966). The mineral mix provided the following (in milligrams per kilogram of feed): calcium phosphate, 735.0; potassium phosphate (dibasic), 81.0; potassium sulfate, 68.0; sodium chloride, 30.6; calcium carbonate, 21.0; sodium phosphate (dibasic), 21.4; magnesium oxide, 25.0; and trace element mix, 18.0. The trace element mix provided ferric citrate, 31.0; zinc carbonate, 4.5; manganese carbonate, 23.4; copper carbonate, 1.85; potassium iodide, 0.04; and citric acid, 39.21.

Hard-shell adult lobsters (400 to 500) were fed each diet ad libitum three times each week for 6 weeks. Growth was determined by change in weight in water (weight of animal suspended in seawater at a salinity of 24‰). This is the method for determining mass changes in slow-growing adult lobsters and use of this technique negates the influence of changes in the animal's water content.

Study 2. The second study was carried out under laboratory conditions to determine the effect of mineral mix source on lobster growth. A basal diet was formulated as described above from conventional feed ingredients according to criteria established earlier (Leavitt et al. 1979; Table 2). No mineral mix was added to the basal diet for treatment 1. Either 10% modified Bernhart-Tomarelli (1966) salt mix (Ca:P = 0.1) or 10% artificial sea salt (Instant Ocean) was added to the basal diet for treatments 2 and 3, respectively. The basal diet had a Ca:P ratio of 0.9:1 and a total mineral content of 5.6%. Each diet was fed ad libitum once a day to five adult lobsters for 5 weeks. Preliminary tests showed that change of weight in water and change of weight in air were comparable measures of change in growth, and growth was therefore measured as the change in weight in air over the 5-week period.

Table 2. Percentage composition of prepared diets for mineral source study.

Ingredient	Basal diet[a]	Supplemented diets	
		Instant Ocean[b]	Modified Bernhart-Tomarelli[c]
Yeast	13.3	12	12
Fish Meal	22.2	20	20
Alfalfa	11.1	10.0	10.0
Flour	52.8	47.5	47.5
Cholesterol	0.6	0.5	0.5
Mineral mix	0.0	10.0	10.0

[a]No supplement.
[b]ICN Nutritional Biochemicals, Cleveland, Ohio (see footnote to Table 1).
[c]Synthetic Sea Salts Aquarium Systems, Mentor, Ohio.

Study 3. The third study examined the effect of supplementation of diets for soft-shell lobsters with calcium orthophosphate (CaH_2PO_4) to increase the levels of calcium and phosphorus. Twelve soft-shell lobsters were placed on each of two diets. The diets, formulated as described above, consisted of the basal unsupplemented diet and one 50% supplemented with CaH_2PO_4 (Table 3). The addition of CaH_2PO_4 to the diet increased its total mineral content without changing the Ca:P ratio. Lobsters were held individually in wooden storage crates at a lobster pound and fed ad libitum three times a week for 6 weeks. Growth was measured as the change of weight in air over the 6-week period.

Table 3. Percentage composition of prepared diets for mineral supplementation study.

Ingredient	No supplement	Supplement
Brewers yeast	5.0	5.0
Fish meal	15.0	15.0
Alfalfa	5.0	5.0
Flour	5.0	5.0
Cholesterol	0.5	0.5
Casein	19.7	19.7
CaH_2PO_4	0.0	50.25
Sand	50.25	0.0

Table 4. Growth of adult lobsters fed diets with different Ca:P ratios (± standard deviation given in parentheses).

Ca:P ratio	No. of lobsters	Weight in water (g)		Weight change (%)
		Initial	Final	
Starvation	10	38.5 (4.1)	36.9 (3.1)	−4.2
0.12:1.0	18	33.5 (5.6)	33.1 (5.6)	−1.2
1.0:1.0	24	35.1 (7.7)	37.3 (6.8)	+6.2
2.0:1.0	23	40.5 (7.4)	37.1 (8.4)	−8.3

Results and Discussion

Study 1. Hard-shell lobsters that were starved or fed prepared diets with Ca:P ratios of 0.12:1 or 2:1 lost weight. Only those lobsters fed the diet with a Ca:P ratio of 1:1 increased mass. The change in mass was greater for lobsters fed a diet with a Ca:P ratio of 1:1 than for those on all other diets. The change in weight was least for lobsters fed a diet with a Ca:P ratio of 2:1. These results (Table 4) are similar to those obtained in studies by Gallagher et al. (1978) with juvenile lobsters, in which growth was significantly better for lobsters on diets with Ca:P ratios of 1:1 or less in comparison with that of lobsters fed diets with Ca:P ratios of 1.5:1 or more. Our results indicated that dietary Ca:P ratios can significantly affect weight gain by adult lobsters.

Study 2. No significant differences were observed when different mineral sources were added to the basal diet of hard-shell lobsters. The basal diet with no added salt mix and the diets with 10% modified Bernhart-Tomarelli salt mix and 10% artificial seawater resulted in weight gains of 1, 0.5, and 1%, respectively (Table 5). The diet with no mineral supplementation supported growth equivalent to that observed with both diets containing mineral mix. This result indicates that dietary mineral supplementation is not necessary in diets formulated from conventional feed ingredients, as long as the Ca:P ratio of the ash in the feed is near 1:1.

Study 3. The two studies described above were conducted with pre-molt hard-shell lobsters caught in the early summer.

Table 5. Growth of adult lobsters fed diets with different salt mixes (± standard deviation given in parentheses).

Salt mix source	Weight (g) Initial	Final	Weight increase (%)
No salt mix	419 (16)	424 (20)	1.0
10% Modified Bernhart-Tomarelli	400 (33)	402 (34)	0.5
10% Instant Ocean	418 (31)	423 (30)	1.0

However, most of the lobsters held in pounds are post-molt, soft-shell lobsters caught in the late summer and early fall. These lobsters are in the process of active recalcification, which is essential to protect the lobster from predators and cannibalism. Previous studies have shown that newly molted lobsters in the wild increase the total amount of minerals and calcium in the diet (Ennis 1973; Leavitt et al. 1979). A final study was therefore conducted to investigate the effect of mineral supplementation on the growth of post-molt lobsters held in pound storage.

No significant differences were observed in the growth of lobsters fed supplemented diets and those fed unsupplemented diets (3.3 and 4.6% increases in weight in air, respectively; Table 6). The calcium and phosphorus levels in the basal diet appeared adequate to support growth and shell recalcification in post-molt lobsters without further supplementation, under pound conditions. Some of the increased mineral found in the gut of wild lobsters in previous studies may be incidentally present and may be ejected as pseudofeces (Williams 1907; Herrick 1909). It is also possible that calcium and phosphorus in the formulated diets were more available for absorption than these elements held in the protein matrix of animals consumed in the wild. The total amount of mineral required by lobsters fed formulated diets may therefore be less than that required by lobsters in the wild.

Several conclusions can be drawn from these studies about the formulation of diets for adult lobsters to be held in pounds:

- The calcium:phosphorus ratios in prepared diets can significantly affect growth (weight gain) in adult lobsters. The Ca:P ratio of 1:1 gave the best growth.

Table 6. Growth of adult lobsters fed diets with different total mineral content (± standard deviation given in parentheses).

Diet	Weight (g) Initial	Final	Weight increase (%)
No supplement	564.6 (71.3)	590.1 (71.4)	4.6
CaH_2PO_4 supplement	548.6 (59.8)	566.2 (63.4)	3.3

- No additional minerals are necessary in semipurified prepared diets formulated from commercial feed sources for maintaining adult lobsters in pounds, as long as the Ca:P ratio in the natural ash of the feed mixture is near 1:1.
- Mineral supplementation of prepared diets fed to soft-shell adult lobsters does not significantly affect weight gain by these lobsters.

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Accepted 24 June 1982

Spawning and Culture of Humpback Chub

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ABSTRACT: During 1981, humpback chub *(Gila cypha)* were spawned and cultured at Willow Beach (Arizona) National Fish Hatchery. This species is listed as endangered by the U.S. Fish and Wildlife Service. Seventeen wild females ovulated after injection with 4 mg acetone-dried carp pituitary per kilogram of body weight. Eight females were stripped manually, whereas the remaining nine injected females spawned naturally in a trout raceway. Mean fecundity of eight females was 2,523. Incubation periods ranged from 102 to 146 h at water temperatures of 21-22 °C, 115 to 160 h at 19-20 °C, 167 to 266 h at 16-17 °C, and 340 to 475 h at 12-13 °C. Survival of eggs was 79% at 21-22 °C, 84% at 19-20 °C, 62% at 16-17 °C, and 12% at 12-13 °C. At hatching, total length and weight of fry ranged from 6.7 to 7.4 mm and 2.8 to 4.2 mg. The survival of swim-up fry was 99% at 21-22 °C, 95% at 19-20 °C, 91% at 16-17 °C, and 15% at 12-13 °C. The length of fry placed in raceways had doubled by 21 to 28 days, and fingerlings attained a range of 36.9 to 47.5 mm and 586.5 to 887.4 mg by day 56 after hatching.

The humpback chub (*Gila cypha*) was one of the last freshwater cyprinids to be discovered in North America (Miller 1946). Although endemic to the Colorado River basin, before 1950 it was known only from the Grand Canyon in Arizona.

The humpback chub is now found sporadically in canyon areas of rivers in the upper and lower basins (Suttkus et al. 1976; Joseph et al. 1977; Suttkus and Clemmer 1977; Behnke and Benson 1980); however, it is considered in danger of extinction. The species is listed as endangered by the States of Arizona, Colorado, Utah, and Wyoming (Deacon et al. 1979) and by the U.S. Department of the Interior (1980).

The purpose of the present study was to develop techniques for the spawning and culture of humpback chub. Data regarding successful spawning of wild brood stock and culture of their progeny during 1981 at Willow Beach (Arizona) National Fish Hatchery are presented.

Methods

Brood stock consisted of 14 wild adults from the Little Colorado River and 16 wild adults from the Colorado River. Fish from the Little Colorado River were collected 17 May 1978, 17 October 1979, and 13 June 1980 near the confluence with the Colorado River, Grand Canyon National Park, Arizona. The Colorado River fish were collected 5 November 1979 at Black Rocks, near Grand Junction, Colorado.

Semi-natural Spawning

Spawning facilities consisted of two concrete raceways (30.5 × 2.4 × 1.2 m) designated as A9 and A10. These raceways were connected to allow water recirculation to obtain the warmer temperatures (at least 18 °C) believed to be necessary for spawning (U.S. Fish and Wildlife Service 1979). Water was pumped to the upper end of A9 from the adjacent raceway (A10) by a 1-hp fresh-flo pump at 0.02 m^3/s, flowed to the lower end, and returned to A10 by gravity. Two layers of spawning substrate were placed in the uppermost 10 m of raceway A9. The bottom layer consisted of boulders (30 to 40 cm) and the top layer of cobble (4-10 cm in diameter). Water introduced at the upper end flowed through the substrate, creating an artificial spawning environment. Water depth over the substrate ranged from 10 to 76 cm.

Nine females and five males (Little Colorado River) were used in this spawning trial. Spawning was induced with intraperitoneal injections of 4 mg acetone-dried carp pituitary per kilogram of body weight (Hamman 1981). Adults were allowed to spawn naturally on the cobble substrate and were removed from the raceway as soon as the eggs began to hatch.

Artificial Spawning

Nine females and seven males (Colorado River) were used in this spawning trial. To induce ovulation, acetone-dried carp pituitary was injected at the rate of 4 mg/kg body weight at 24-h intervals. After receiving the injection, fish were placed in a holding tank (6.0 × 0.75 × 0.75 m) until they could be manually stripped. Warm water (21-22 °C) flowed through the tank at 23 L/min. When eggs could be expressed, a female was manually stripped and the eggs fertilized with milt from one male. After water hardening (50 min), eggs were poured on screen trays (53 × 53 cm) covered with 1.4-mm-mesh hardware cloth, and then placed in four aluminum troughs (3.5 × 0.55 × 0.3 m) and slanted at a 30° angle into the incoming water. To determine incubation periods at different water temperatures, warm water (21-22 °C) entered two troughs, cool water (16-17 °C) entered one trough, and cold water (12-13 °C) entered the fourth trough, all at 8 L/min.

Table 1. Total lengths and weights of wild humpback chubs (of unknown age) held at Willow Beach (Arizona) National Fish Hatchery as of 5 May 1981.

Fish stock	No.	Length (mm) Mean	Length (mm) Range	Weight (g) Mean	Weight (g) Range
Little Colorado River					
Females	9	395	372-425	588	494-730
Males	5	355	315-378	329	260-470
Colorado River					
Females	9	382	355-409	507	350-730
Males	7	357	327-378	370	268-460

Table 2. Summary of data for manually stripped eggs of nine female humpback chub at Willow Beach (Arizona) National Fish Hatchery, 1981.

Fish		Eggs	
Total length (mm)	Weight (g)	Fecundity (No. of eggs)	Fertilization (%)
409[a]	730	0	0
387	540	3,520	80
406	690	5,445	77
401	560	3,795	92
361	400	1,485	46
390	500	2,805	83
372	416	1,925	85
355	350	880	57
359	380	330	0

[a]Fish received three injections.

Rearing Facilities

The rearing facilities consisted of two recirculating systems or four concrete raceways (30.5 × 2.4 × 1.2 m) that were designated as A9 and A10, and C1 and C2. Water was recirculated with a ½-hp fresh-flo pump at 0.01 m^3/s. The raceways were filled 1 week before spawning (except A9 and A10) to obtain warmer water temperatures; fertilizers (ammonium phosphate and commercial trout starter) were added to produce a plankton bloom. Zooplankton, predominately cladocerans, were observed in 10 days.

Measurements of egg diameters, newly hatched fry, and fingerlings were made to the nearest 0.1 mm, and weights were obtained to the nearest 0.1 mg. The number of eggs spawned naturally by wild fish on the cobblestone substrate were estimated by visual observations; estimates of number of fry hatched were based on the fertility rate of the eggs.

Results

Males exhibited orange to red-orange breeding colors on the pectoral and pelvic fins, anal fin, and entire lower sides when spawning began (5 May 1981; water temperature 19 °C). Females were light orange on the sides and at the base of the pectoral and pelvic fins and anal fin. Females were normally larger than the males (Table 1). Both sexes had fine tubercles on the head, operculars, and paired fins. All males produced semen with active sperm; however, no eggs could be expressed from the females until after hormone injections.

Spawning

Semi-natural: Nine females spawned (5-6 May 1981) within 24 h of the first injection. The spawning area covered 1.0 × 2.4 m in a water depth of 35 to 45 cm; water temperatures were 19-20 °C. An estimated 30,000 eggs were spawned naturally by the nine females. Egg diameters varied from 2.6 to 2.9 mm (mean, 2.8 mm). Since all the eggs adhered to the cobble, they were allowed to hatch on the substrate.

Artificial: Eight females ovulated (1-4 June 1981) 20 h after the first injection; one female failed to ovulate after receiving three injections. Egg diameters ranged from 2.6 to 2.8 mm (mean, 2.7 mm); volumetric measurements varied from 51 to 58 eggs/mL (mean, 55 eggs/mL). Total fecundity was 20,185 (mean, 2,523/female) or 5,262 eggs per kilogram of body weight (Table 2).

Incubation and Hatching

Hatching began in raceway A9 (water temperature 19-20 °C) at 115 h and was complete at 160 h; total length of newly hatched fry averaged 7.1 mm (Table 3). Eggs placed on the slanted screen trays in water temperatures of 21-22, 16-17, and 12-13 °C began hatching at 102, 167, and 340 h, respectively, and hatching was complete at 146, 266, and 475 h, respectively; total lengths of these newly hatched fry averaged 6.9 mm (Table 3).

Culture

When fry began swimming to the surface in the hatching troughs, 72 to 168 h after hatching, 12,381 were transferred to raceways C1 and C2. The 23,490 fry that hatched naturally on cobblestone substrate were cultured in the same raceways (A9 and A10).

During the first 2 weeks the fry were very active and fed near the surface. Schooling behavior was observed throughout the present study.

On day 28, zooplankton in the raceways began declining and a dry commercial trout starter was fed. The fry were fed 4 to 5 times daily (0.25 to 0.50 kg of feed per system per day). By day 35, when the fry averaged 22.4 and 32.9 mm total length, most fish accepted the trout starter diet. On several occasions, river water (12-13 °C) was introduced to the recirculating systems to compensate for evaporation and to improve water quality. This addition of cold water usually lowered the temperature 7 Celsius degrees in 2 h. Benthic feeding behavior was observed at reduced water temperature, but as the temperature increased, the fish resumed feeding near the surface.

Table 3. Summary of data for incubating eggs of humpback chub at four different water temperatures. All eggs were artificially fertilized except those incubated at 19-20°C.

Incubation data	Water temperature (°C)			
	21-22	19-20	16-17	12-13
Number of eggs	19,910[a]	30,000[b]	165	110
First cleavage (h)	3	3	4	6
Embryo developed (h)	70	75	105	205
Hatching began (h)	102	115	167	304
Hatching ended (h)	146	160	266	475
Percent hatch (%)	79	84[b]	62	12
Numbers hatched	15,207[c]	25,200[b]	102	13
Age of swim-up fry (h)	72	72	120	168
Percent swim-up (%)	99	95[b]	91	15
Number of fry	12,381	23,940[b]	93	2

[a]A total of 660 eggs were transferred to Utah State University at Logan.
[b]Estimated numbers.
[c]A total of 2,700 fry were transferred to the University of Idaho at Moscow.

Continuous water temperature recordings were made and carbon dioxide, ammonia nitrogen, dissolved oxygen, and pH were analyzed each week to monitor water quality during culture. Water temperature ranged from 12.8 to 25.5°C, carbon dioxide from 2.11 to 4.00 mg/L, ammonia nitrogen from 0.01 to 0.59 mg/L, dissolved oxygen from 9.1 to 10.6 mg/L, and pH from 7.9 to 8.75 units.

Samples of fry were collected each week to determine growth (Table 4). On day 56, 19,875 fingerlings (mean, 36.9 mm and 588.5 mg) were removed from raceways A9 and A10 (semi-naturally spawned), and 9,318 fingerlings (mean, 47.5 mm and 887.4 mg) were removed from raceways C1 and C2 (artificially spawned); survival rates were 83 and 75%, respectively. The fish increased 5- to 7-fold in total length during 56 days of culture.

Discussion

Suttkus and Clemmer (1977) stated that humpback chubs probably spawn during June and July in the Grand Canyon, Arizona, whereas Holden (1978) indicated that spawning may occur during May and June in the Desolation Canyon, Utah. Their observations were based on the collection of gravid females, ripe males, and juvenile fish from the Little Colorado River, Arizona, and the Green River, Utah. During the present study, spawning occurred in May (Little Colorado River fish) and June (Colorado River fish) when water temperatures were 19-20 and 21-22°C, respectively.

The decline of the humpback chub has been attributed to loss of habitat created by dams, reduced flows below cold and fluctuating tailwaters of dams, and hybridization (Vanicek et al. 1970; Holden and Stalnaker 1970, 1975; Minckley 1973; Smith et al. 1979). The 88% egg mortality and 85% fry mortality hatched at low temperatures (12-13°C) support the theory of dam-related decline, since similar temperatures are common below dams. Hybridization may also occur, because the bonytail chub *(Gila elegans)* has been artificially hybridized with humpback chub in a hatchery (Hamman 1981).

The future of the humpback chub may depend on hatchery efforts to spawn and culture the species, and reintroduction of hatchery-reared progeny in the wild. Methods described in the present study appear to be suitable for full-scale propagation, to assist in restoration of this endangered species.

Acknowledgments

I thank Lyle Miller, Peter Carboni, Joe Saenz, and the staff at Willow Beach National Fish Hatchery for assistance during the study, and Theophilus Inslee, Buddy Jensen, J. Mayo Martin, John Mazuranich, Joseph McCraren, William Miller, and Robert Piper for reviewing the manuscript.

Table 4. Total lengths and weights of larval humpback chubs cultured during 1981 at Willow Beach (Arizona) National Fish Hatchery.

Day	Little Colorado River Progeny				Colorado River Progeny			
	Total length (mm)		Weight (mg)		Total length (mm)		Weight (mg)	
	Mean[a]	Range	Mean[a]	Range	Mean[a]	Range	Mean[a]	Range
0[b]	7.1	6.8- 7.4	3.9	3.8- 4.2	6.9	6.7- 7.3	3.5	2.8- 3.7
7	10.2	10.0-10.6	5.1	4.7- 5.7	9.6	9.0- 9.8	4.7	4.9- 5.4
14	10.7	10.0-11.8	6.9	5.5- 10.6	11.5	10.8-12.9	9.7	7.2- 15.4
21	12.0	10.8-14.8	14.3	9.6- 21.4	17.1	13.6-18.4	43.2	15.6- 54.0
28	15.2	10.8-19.8	33.8	18.6- 78.2	24.9	22.7-27.2	144.9	115.8- 188.3
35	22.4	18.9-25.7	101.0	59.0-152.1	32.9	30.0-35.1	358.3	298.4- 448.4
42	30.3	22.9-35.7	309.1	140.4-427.9	38.6	34.0-40.9	525.8	397.6- 649.2
49	33.3	30.1-37.7	393.2	304.6-531.8	41.4	35.9-45.9	670.6	540.0- 920.1
56	36.9	30.3-44.2	588.5	361.6-923-2	47.5	43.2-51.1	887.4	720.5-1,006.4

[a]Mean total length and weight of 10 fish per sample.
[b]Age of newly hatched larvae.

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Accepted 2 April 1982

Growth of Fed and Unfed Bighead Carp in Cages at Two Stocking Densities

Little research has been done in the United States on production of bighead carp *(Aristichthys nobilis)*, although preliminary marketing work indicates high potential acceptance (Engle 1978). Traditionally, bighead carp have been extensively cultured in polyculture ponds in Asia (Bardach et al. 1972). Although the principal foods of bighead carp are zooplankton and detritus (Lazareva et al. 1977), stomach analyses have shown that artificial feeds may also be consumed (Cremer and Smitherman 1980). The objectives of the present study were to determine if production of bighead carp could be increased by providing artificial feed and if cage culture would be feasible for bighead carp production.

Twelve cylindrical cages, constructed of 6.4-mm-mesh plastic-coated hardware cloth and floated with Styrofoam packing material, were suspended in a line at 1-m intervals in a 0.6-ha pond. Water volume in each cage was about 0.45 m^3. Treatments with and without feed at fish densities of 300 and 600/m^3 were each replicated three times and averages reported. A table of random numbers was used to assign treatments to cage locations in the pond and analysis of variance was used to determine statistical significance among treatments and intertreatment effects.

Fingerling bighead carp averaged 36 g when stockd on 1 July 1979. Cages were stocked randomly from a single pool of fish. The fish in the cages that received feed were fed once daily 7 days a week with a floating catfish feed containing 35% crude protein (Producer's Feed Company, Belzoni, Mississippi) at a rate of 3% of body weight per day for 72 days. Samples of a minimum of 20% of the fish in each cage were counted and weighed every 2 weeks and the averaged weight was used to adjust feeding rates for the next 2-week period. Fish were harvested after 72 days and number and weight of fish were recorded. One group had a 23% mortality and these data were discarded.

Although fish ingested the pellets, feeding was limited. Fish that were fed grew more rapidly than fish that were not fed ($P < 0.01$), but growth was poor in both groups (Table 1). Stocking density had no discernible effects on fish growth in either treatment ($P > 0.05$; Table 1). In 72 days the average weight of fish that were fed increased from 37 to 51 g (38%), but feed conversion was poor (S = 8). However, the average weight of fish that were not fed also increased from 34 to 42 g (24%) — a result that would not have been expected of filter-feeding fish confined to a cage in a static pond.

Table 1. Mean growth, survival, and feed conversion of fed and unfed bighead carp in cages at two stocking densities, Auburn, Alabama, 1979.

Treatment	Stocking		Harvest		Production		Feed	
	Density (fish/m³)	Total weight (kg)	Survival (%)	Total weight (kg)	Net per cage (kg)	Average daily (g)	Total fed (kg)	Conversion (S)[a]
Fed[b]	600	9.9	98	12.9	3.0	0.16	26.0	8.8
	300	5.0	97	7.1	2.0	0.23	14.9	7.7
Unfed	600	9.0	98	10.7	1.7	0.10	—	—
	300	4.8	99	5.8	1.0	0.11	—	—

[a]S conversion = dry weight of feed fed/wet weight gain of fish (Swingle 1967).
[b]Data from one replicate discarded because of 23% mortality.

In contrast to results for cage production of channel catfish (*Ictalurus punctatus;* Schmittou 1969) and tilapia (*Tilapia* spp.; Galbreath 1979), the poor feed conversion and growth and the opportunity cost of producing bighead carp in cages at densities of 300 and 600/m³ would make this system unprofitable for fish farmers. Furthermore, although fish grew faster with feed than without feed, they did not reach the size (1-2 kg) considered marketable in the United States (Engle 1978). Bighead carp are apparently unable to use floating catfish feed efficiently. Nutritional research is needed to determine the digestibility of alternative feedstuffs for bighead carp (such as alfalfa pellets, processed swine wastes, or other agricultural by-products) that might improve feed conversion and economic feasibility.

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Accepted 26 February 1982

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Infectious Hematopoietic Necrosis: A Comparison of Epizootics Among Fry Hatched from Saline-rinsed and Unrinsed Steelhead Eggs

Epizootics of the viral disease infectious hematopoietic necrosis (IHN) occurred in fry of summer steelhead trout (*Salmo gairdneri*) at Round Butte Hatchery (financed by Portland General Electric Company and operated by the Oregon Department of Fish and Wildlife) during 1975, 1976, 1978, and 1980. In most years, some production lots of fry did not become diseased even though they were derived from the same parents and held under identical conditions, but in a different container. Although infectious virus has been detected in the brood stock most years, there has been little correlation between detection of the virus in adults and epizootics in their progeny. Epizootics are typically catastrophic, with more than 90% mortality in 3 weeks. Current practice is to destroy all fry in the rearing container when mortality increases and IHN virus is identified (McDaniel 1979).

Although vertical transmission is suggested when a high carrier rate is observed in adults, the possibility of horizontal transmission by means of the hatchery water source also exists. Round Butte Hatchery is located immediately below Round Butte Dam on the Deschutes River, a tributary of the Columbia River in central Oregon. Spring water (constant 10°C) resulting from seepage out of the resevoir (Lake Billy Chinook) above is used. Landlocked sockeye salmon (*Oncorhynchus nerka*) in this reservoir are known to carry IHN virus (Mulcahy et al. 1980).

To maintain production while anticipating epizootics, 250,000 eggs (three times the number needed to meet production goals) are taken from each of three adult-run segments, early, middle, and late, differentiated by trap-entry times. Other physical measures to reduce the impact of epizootics include separating the incubator stacks (some epizootics have occurred while the fry were still in the incubators) and increasing the number of starting tanks sixfold. These measures decrease the percentage of the production in each rearing container and consequently reduce the number of fish that must be destroyed when epizootics occur.

Salt solutions have been used for many years as a medium for separating dead eggs from living, eyed eggs on the basis of differences in density (Leitritz and Lewis 1976). Salt solutions have also been used in egg-taking to keep the albumen from broken eggs in solution and to prevent agglutination of sperm, thus increasing fertilization (Leitritz and Lewis 1976). Wingfield (1972) used a saline solution to force eggs from salmonids, thus increasing production. It was this later application that stimulated this experiment, conducted in 1979 and 1980, with the objective of determining whether removal of ovarian fluid from steelhead eggs before fertilization by rinsing them with a saline solution would decrease the incidence of IHN in the resulting fry. We hypothesized that removing ovarian fluid would decrease the number of virus particles entering the eggs during fertilization and water-hardening and, as a result, reduce the probability of an epizootic. A second goal of the study was to compare the survival of rinsed and unrinsed groups of eggs and fry.

For each trial, about 18 females were spawned, their eggs were pooled, and ovarian fluid was drained from the eggs. About one-half of the eggs were then poured into another container and one-half of the ovarian fluid was returned to these eggs. The eggs without ovarian fluid were then rinsed three times, using 23 L of 1% NaCl. One-half of the pooled milt from 20 males was added to each egg pool. Water was added to activate sperm and achieve fertilization. After about 30 s, the fertilized eggs were rinsed with fresh water and then allowed to water-harden for 1 h before disinfection with an organic iodine solution (Wescodyne, 1:150 for 10 min, 100 ppm active iodine) and transport to the hatchery. Rinsed and unrinsed subgroups were placed in separate Heath-incubator stacks. After hatching, each lot of fry was placed in a separate 1.8-m circular tank, and losses of eggs and fry were recorded. Proportions of rinsed and unrinsed subgroups surviving to stocking in ponds were compared by using the normal deviate z (Snedecor and Cochran 1967) to determine the significance of differences.

Three trials were conducted in 1979 with fish from the late part of the run. All adults were assayed (ovarian fluid and milt) for the presence or absence of IHN virus (McDaniel 1979). Eight trials were conducted in 1980: three each with fish from the early and middle segments of the run and two from the late segment. Equipment was disinfected between trials. Sampling for infectious virus during egg-takes in 1980 was limited to ovarian fluid from 45 females of the middle group. Ovarian fluid from both rinsed and unrinsed pooled subgroups (middle egg-take) was also sampled immediately before the introduction of milt. Fry were sampled for IHN virus soon after any abnormally high mortality was observed. If virus was identified, the fry were destroyed.

Virus Sampling during Spawning. No IHN virus was found in samples taken from adults spawned during 1979 (Table 1). In 1980, however, 44% of the samples from the middle group harbored IHN virus. Samples from both rinsed and unrinsed subgroups in two of the three pooled groups tested (7 and 8) also contained IHN virus (Table 1). Cytopathic effect in cell culture monolayers appeared later and was more diffuse in the rinsed than in the unrinsed subgroups, suggesting that fewer virus particles were present in the rinsed subgroups. However, since no quantification of the virus was attempted, comparisons are difficult.

Survival to Stocking in Ponds. Epizootics of IHN did not occur during 1979 or 1980 before the fish were stocked in ponds. During 1979, survival to stocking was significantly better ($z > 24$; $P < 0.01$) in the rinsed subgroups than in the unrinsed (Table 1). In 1980, however, nearly the opposite was found; survival to stocking was significantly higher for the unrinsed subgroups in six of the eight comparisons. Pooling all groups, survival to stocking was similar when comparing eggs that were rinsed (68.3%) with those that were not rinsed (67.8%). However, more variation in survival to stocking was seen among the rinsed (SD = 15.3%) than among the unrinsed (SD = 9.2%) subgroups.

IHN Epizootics after Stocking. No IHN epizootics occurred in the three trials with the late egg-take group in 1979 (Table 1). In 1980, epizootics occurred in five of the eight rinsed subgroups and in none of the unrinsed subgroups. Because pooled ovarian fluid was sampled for IHN virus in only three of the eight trials in 1980, it is not known which groups were fertilized and water-hardened in the presence of the virus. Comparing only those five trials in which epizootics occurred, however, the probability that only rinsed subgroups would develop IHN through chance alone is very low ($P = 0.03$).

In the present experiment, survival to stocking comparing rinsed and unrinsed subgroups was inconsistent. At Round Butte Hatchery, survival has historically been poorer in fish from the early and middle run segments than from the late segments. We have attributed this difference to a higher percentage of overripe females in the early and middle groups. Differences in egg durability between egg-take (brood) groups may have caused the observed differences in

Table 1. Survival to stocking in ponds and incidence of IHN epizootics in saline-rinsed (R) and unrinsed (U) groups of steelhead trout at Round Butte Hatchery in 1979 and 1980.

Brood group, trial no., and no. of fish	No. of eggs and treatment	Virus detected after treatment	Fry		
			Survival (%)	Date	
				Stocked	IHN epizootic
1979 Late					
1 20	56,640 R	No	82.2		—
	48,952 U	No	53.4		—
2 20	54,438 R	No	85.8	19 April	—
	50,007 U	No	74.2		—
3 20	60,135 R	No	77.7		—
	57,603 R	No	71.5		—
1980 Early					
4 18	48,000 R		67.5		1 April
	47,280 U		63.2		—
5 18	47,520 R	No Data	54.5	18 March	—
	47,520 U		56.3		—
6 18	51,360 R		63.6		1 April
	46,560 U		67.0		—
1980 Middle					
7 18	48,144 R	Yes	64.0		5 May
	43,424 U	Yes	74.9		—
8 18	46,020 R	Yes	58.8	25 March	—
	38,940 U	Yes	81.5		—
9 10	35,872 R	No	36.1		27 May
	25,016 U	No	55.4		—
1980 Late					
10 18	44,000 R		61.7		—
	45,000 U		64.5		—
		No Data		22 April	
11 18	40,384 R		87.6		12 May
	47,424 U		73.5		—

the present experiment. In four of the five trials from the late groups (probably the highest quality eggs), rinsing with the saline solution increased fertilization, increased survival of the embryos and fry, or both. Conversely, in five of six trials with eggs of the early and middle groups, survival was poorer after the eggs had been rinsed, perhaps because a larger percentage of these eggs were unable to survive the increased agitation during rinsing and draining.

The IHN virus was not found in samples taken from adults spawned in 1979, and this was consistent with the absence of IHN in fry derived from those spawns. In 1980, however, IHN virus was found in ovarian fluid from pooled groups 7 and 8 of the middle brood, but not from group 9, whereas IHN epizootics occurred in the rinsed 7 and 9 groups but not in group 8. The virus causing the epizootic in group 9 may have come through the water supply. However, because IHN virus was found in 44% of the females tested from the middle group, it is likely IHN was present in group 9 but was not detected.

Saline rinsing of salmonid eggs during artificial spawning has potential application for reducing the number of maternally produced pathogens in the egg environment

before fertilization. The probability for vertical transmission of pathogens small enough to enter the egg during fertilization and water-hardening may be reduced by such rinsing. Although the saline rinse would seem to have served this purpose in the present experiment, our results suggest that an unknown factor led to disease in the fry of some rinsed groups.

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Accepted 5 March 1982

Portable Trap for Collecting Adult Sea Lampreys

Lampricides, TFM (3-trifluoromethyl-4-nitrophenol) and Bayer 73 (5,2′-dichloro-4′-nitrosalicylanilide), have been used successfully to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes (Smith and Tibbles 1980). The effectiveness of control is assessed in part by the number and biological characteristics of adults captured during the spawning migration.

Many methods have been devised to capture spawning-run lampreys. Wicker baskets placed in riffles were used to harvest sea lampreys in Canada (McCauley 1960) and river lampreys (*Lampetra fluviatilis*) in Europe (Sjoberg 1980; Tuunainen et al. 1980; Valtonen 1980). In the United States, dip nets (Applegate and Smith 1951), gill and trap nets (Smith and Elliott 1953), wooden-framed traps and barrier nets (Wigley 1959), and mechanical and electrical barriers (McLain et al. 1965) have been used to collect adult sea lampreys in Great Lakes tributaries. These methods were either inefficient in capturing a large proportion of the spawning run or were expensive to operate and maintain. A trapping device was needed that would require little maintenance yet efficiently capture these upstream-migrating adults. We describe the design and use of a portable trap which captures adult sea lampreys at dams and natural barriers.

The trap consists of an iron frame covered with galvanized hardware cloth (13-mm mesh) and with a funnel at each end. Top and bottom panels are constructed of 2.5-cm angle iron welded to form a rectangle, 117 × 61.4 cm (Fig. 1); hardware cloth is riveted inside the frame. Each side is made of hardware cloth riveted between two rectangles (116 × 46.2 cm) constructed of strap steel (0.3 × 1.3 cm). The top,

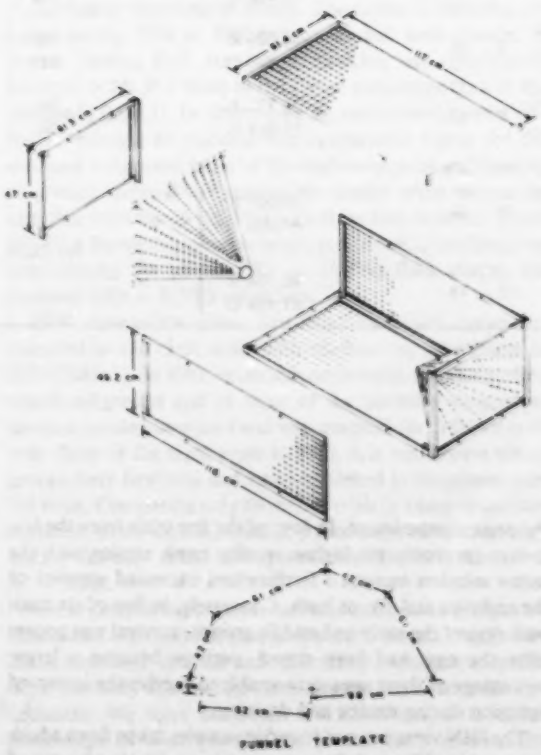

Fig. 1. Diagram of a portable trap and funnel template.

Fig. 2. Placement of the trap along the face of a dam.

bottom, and sides lie flat during transport and are easily assembled with eight bolts at the trapping site.

Funnels are made of hardware cloth, cut and folded as illustrated by the template (Fig. 1). A funnel opening 7.6 cm in diameter is cut and a 0.6-cm length of polyvinyl chloride tubing of the same diameter is attached with hog rings to provide a smooth, rigid opening. Each funnel is riveted to a frame (61.5 × 47 cm) made of strap steel and angle iron. Oversized holes in the funnel frame pass over two bolts tapped through each end of the top and bottom panels of the trap. The funnels project inward and toward the bottom of the trap. One funnel is secured with wing nuts and can readily be removed to empty the trap.

Adult sea lampreys often congregate at dams and natural barriers during upstream migration. The trap is placed adjacent to a barrier, flush against a vertical wall, preferably near a spillway. The device is suspended by a chain (Fig. 2) or, in shallow areas, is rested on the stream bottom. When properly positioned, all but the upper 10 cm are submerged. The weight of the trap (40.8 kg) helps maintain its position in swift current. Walls serve as a lead and guide lampreys into traps as they probe the barrier for an upstream passage. In most streams a trap can be removed by two persons; however, block and tackle may be required when many lampreys are captured.

From 1975 to 1980 portable traps were fished in 76 U.S. tributaries of the Great Lakes to assess adult sea lamprey runs and to test the device under various conditions. Traps were fished for several years at sites where many lampreys were collected but for one spawning season where few or none were collected. A total of 70,711 adult sea lampreys were captured. Mark and recapture studies indicated that the traps were most efficient at a barrier near the mouth of a stream (Braem and Moore 1977, 1979). At most stations traps were emptied five times a week; lampreys were usually captured at night. Mortality of lampreys and other fishes was negligible. The funnel opening allowed entrance only to lampreys and small fishes (< 25 cm long), mostly suckers and minnows.

The traps are presently being used by sea lamprey control units in the United States and Canada and have been placed at suitable barriers throughout the Great Lakes. In 1979, 30 of the most productive U.S. tributaries were chosen as index sites to assess adult sea lamprey runs. Traps will fish at these sites throughout the annual spawning run, normally April to June.

Problems of vandalism and theft of the traps have occurred in urban areas, but the advantages of these simple devices outweigh the problems. Traps are inexpensive (cost of materials was about $200 in 1980) and the collapsible design permits easy transport. They have been successfully fished where flows were as low at 0.06m³/s (Trout River, Presque Isle County, Michigan) and as high as 2,123 m³/s (St. Marys River, Chippewa County, Michigan). The portable traps require little maintenance and are an efficient and economical means of assessing adult sea lamprey spawning runs in tributaries of the Great Lakes.

Acknowledgments

We thank Harold A. Purvis for his guidance of the project, and Jeff Key, who prepared the illustration.

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Accepted 5 March 1982

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